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(54) Title: G–PROTEIN COUPLED HEPTAHELICAL RECEPTOR BINDING COMPOUNDS AND METHODS OF USE THEREOF

(57) Abstract

G–protein coupled heptahelical binding compounds are described, e.g. compounds of the formula (I): J–M wherein J is an aromatic moiety and M is a G–protein coupled heptahelical receptor pocket interacting moiety. The compounds of the invention can be used to treat chemokine mediated disorders, e.g. neurological, immunological, inflammatory and cancer related disorders.

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G-PROTEIN COUPLED HEPTAHELICAL RECEPTOR BINDING COMPOUNDS AND METHODS OF USE THEREOF

Background of the Invention

The chemokine family of peptides is defined on the basis of sequence homology and on the presence of variations on a conserved cysteine motif (Schall (1996) *Cytokine* 3:165-183; and Oppenheim et al. (1991) *Annu. Rev. Immunol.* 9:617-648). The family can be subdivided on the basis of this motif into two major subfamilies, in which members of each contain four characteristic cysteine residues. This subdivision therefore defines the CC or β-chemokine family in which the first two cysteines are juxtaposed, and the CXC or α-chemokine family in which there is an intervening amino acid between the first two cysteines. Two other subfamilies have subsequently been described which have variations in the number of amino acids between the first two cysteine residues (Kelner et al. (1994) *Science* 266:1395-1399; Dorner et al. (1997) *J. Biol. Chem.* 272:8817-8823; and Bazan et al. (1996) *Nature* 385:640-644.)

Chemokines display a range of *in vitro* and *in vivo* functions ranging from proinflammatory activities on a range of cell types to proliferative regulatory activities. All of the functions of the chemokine family are believed to be signaled into a responsive cell using members of the G protein-coupled heptahelical receptor family. To date several α-chemokine and β-chemokine receptors have been described. (e.g., Neote et al. (1993) *Cell* 72:415-425; Ponath et al. (1996) *J. Exp. Med.* 183:2437-2448; and Power et al. (1995) *J. Biol. Chem.* 270:19495-19500.)

Summary of the Invention

The present invention is based, at least in part, on the discovery of compounds which interact with G-protein coupled heptahelical receptors. The compounds of the invention can be used to treat chemokine mediated disorders, e.g. neurological, immunological, inflammatory and cancer related disorders.

The present invention provides a G-protein coupled heptahelical receptor (GPCR) binding compound of the formula:

J-M (I)

where J is an aromatic moiety and M is a G-protein coupled heptahelical receptor pocket interacting moiety. Preferably, the compound interacts with a β-chemokine receptor, e.g. CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, or CCR10. In a preferred aspect, the compound modulates recruitment of at least one cell type, e.g. leukocytes, e.g. macrophages or eosinophils, associated with inflammation in a subject.

In another aspect, the invention pertains to a method for treating a chemokine mediated disorder in a subject. The method involves administering an effective amount of a G-protein coupled heptahelical receptor binding compound such that the disorder is treated, e.g. at least one symptom of the disorder is diminished or alleviated. The chemokine mediated disorder may be a neurological disorder (e.g. multiple sclerosis, Alzheimer's disease, or Parkinson's disease), an immunological disorder (e.g. AIDS, arthritis, or lupus), cancer, or an inflammatory disorder (e.g. asthma).

In another aspect, the invention pertains to a compound represented by the formula:

$$A\text{-}L_1\text{-}B\text{-}L_2\text{-}E \qquad (II)$$

wherein

A is selected from the group consisting of branched and straight chain alkyl, aryl, alkenyl, alkynyl, and heteroaryl moieties optionally substituted by NR'R", CN, NO_2, F, Cl, Br, I, CF_3, CCl_3, CHF_2, $CHCl_2$, CONR'R", S(O)NR'R", CHO, OCF_3, $OCCl_3$, SCF_3, $SCCl_3$, COR', CO_2R', and OR' and wherein R' and R" are each independently hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, or optionally substituted aryl;

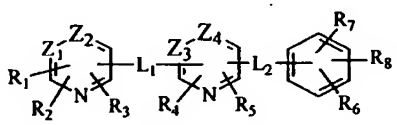
L_1 is a linker moiety selected from the group consisting of a bond, O, S, CHOH, CHSH, $CHNH_2$, CHNHR, CHNRR', NH, NR, $(CH_2)_n$, $O(CH_2)_n$, and $(CH_2)_nO(CH_2)_n$, an optionally substituted ring moiety of 4 to 7 atoms containing up to three heteroatoms, a chain of 1 to 5 atoms optionally substituted by C_1-C_6 alkyl, halogens, wherein n is either 0, 1, 2, or 3, and R and R' are each independently substituted or unsubstituted C_1-C_6 branched or straight chain alkyl, C_1-C_6 branched or straight chain alkenyl, aryl, C_4-C_7 ring, optionally substituted with up to three heteroatoms;

B is an aromatic moiety containing from 0 to 3 heteroatoms and containing 5 to 7 members optionally substituted by NR'R", cyano, nitro, halogen, CF_3, CHF_2, CONR'R", S(O)NR'R", CHO, OCF_3, SCF_3, COR', CO_2R', OR' where R' and R" are each independently hydrogen, halogen, C_1-C_6 alkyl, optionally substituted aryl or optionally substituted aryl;

L_2 is a second linking moiety selected from the group consisting of a bond, $CH_2C=O$, NHC=O, OC=O, C=O, $CH_2NHC=O$, CHOH, $(CH_2)_n$, O, NH, $O(CH_2)_n$, $NH(CH_2)_n$, CH_2CHOH and NRC=O; and

E is a G-protein coupled heptahelical receptor pocket interacting moiety.

In another aspect, the invention also relates to a compound represented by the formula:



(III)

wherein

Z_1, Z_2, Z_3, and Z_4 are each independently N or C;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, C_1-C_6 branched or straight chain alkyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl;

L_1 is O, S, NH, NR_7, $(CH_2)_n$, CO, CHOH, $O(CH_2)_n$, and $(CH_2)_nO(CH_2)_n$ wherein n is either 1,2, or 3 and R_7 is C_1-C_6 branched or straight chain alkyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl; and

L_2 is a second linking moiety selected from the group consisting of a bond, $CH_2C=O$, NHC=O, OC=O, C=O, $CH_2NHC=O$, CHOH, $(CH_2)_n$, O, NH, $O(CH_2)_n$, $NH(CH_2)_n$, CH_2CHOH and NRC=O.

In yet another aspect, the invention relates to a pharmaceutical preparation comprised of the G-protein coupled heptahelical receptor binding compound and a pharmaceutically acceptable carrier. The invention also pertains to a packaged G-protein coupled heptahelical receptor binding compound containing instructions for using the compound for treating a chemokine mediated disorder.

Brief Description of the Drawings

Figure 1 depicts a binding curve of Compound A in a DB Assay (see Example 3 below).

Figure 2 depicts a binding curve of Compound CU in a DB Assay (see Example 3 below).

Figure 3 depicts a binding curve of Compound CV in a DB Assay (see Example 3 below).

Figure 4 is a graph depicting the blockage of THP.1 cell migration by compound B in a CBIR Assay (see Example 4 below).

Figure 5 is a graph depicting the blockage of THP.1 cell migration by compound C in a CBIR Assay (see Example 4 below).

Figure 6 shows MCP-5 induced peritoneal eosinophil recruitment in mice after administering compounds B and C in a MIR Assay (see Example 5 below).

Detailed Description of the Invention

The present invention pertains to a G-protein coupled heptahelical receptor binding compound of the formula:

J-M (I)

wherein J is an aromatic moiety and M is a G-protein coupled heptahelical receptor pocket interacting moiety.

The language "G protein coupled heptahelical receptor" includes receptors for which belong to the G-protein coupled receptor (GPCR) superfamily of seven-transmembrane domain heptahelical receptors. G-protein coupled receptors (GPCRs), along with G-proteins and effectors (intracellular enzymes and channels which are modulated by G-proteins), are the components of a modular signaling system that connects the state of intracellular second messengers to extracellular inputs. These genes and gene-products are potential causative agents of disease (Spiegel et al. (1993) *J. Clin. Invest.* 92:1119-1125; McKusick and Amberger (1993) *J. Med. Genet.* 30:1-26). Specific defects in the rhodopsin gene and the V2 vasopressin receptor gene have been shown to cause various forms of autosomal dominant and autosomal recessive retinitis pigmentosa (see Nathans et al. (1992) *Annu. Rev. Genet.* 26:403-424), nephrogenic

diabetes insipidus (Holtzman et al. (1993) *Hum. Mol. Genet.* 2:1201-1204 and references therein). These receptors are of critical importance to both the central nervous system and peripheral physiological processes. Evolutionary analyses suggest that the ancestor of these proteins originally developed in concert with complex body plans and nervous systems.

The GPCR protein superfamily now contains over 250 types of paralogues, receptors that represent variants generated by gene duplications (or other processes), as opposed to orthologues, the same receptor from different species. The superfamily can be broken down into five families: Family I, receptors typified by rhodopsin and the beta2-adrenergic receptor and currently represented by over 200 unique members (reviewed by Dohlman et al. (1991) *Annu. Rev. Biochem.* 60:653-688 and references therein); Family II, the recently characterized parathyroid hormone/calcitonin/secretin receptor family (Juppner et al. (1991) *Science* 254:1024-1026; Lin et al. (1991) *Science* 254:1022-1024); Family III, the metabotropic glutamate receptor family in mammals (Nakanishi (1992) *Science* 258:597-603); Family IV, the cAMP receptor family, important in the chemotaxis and development of *D. discoideum* (Klein et al. (1988) *Science* 241:1467-1472); and Family V, the fungal mating pheromone receptors such as STE2 (reviewed by Kurjan (1992) *Annu. Rev. Biochem.* 61:1097-1129).

Examples of GPCRs include chemokine receptors which are expressed in specific tissues and leukocyte subtypes. Many chemokine receptors can bind to and be activated by more than one chemokine, and many chemokines can bind and activate more than one receptor in the nanomolar or subnanomolar range (MacKay (1996) *J. Exp. Med.* 184:522-549; Wells et al. (1996) *Chem. Biol.* 3:603-609). Chemokine receptors can be classified generally into three groups: α-chemokine receptors, β-chemokine receptors and α-β chemokine receptors. Preferably, the GPCR binding compounds of the present invention interact with receptors of the β-chemokine receptor family (Bonini et al. *DNA Cell Biol.* (1997) 16(10):1249-1256). Examples of β-chemokine receptors include CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, and CCR10.

The β-chemokine receptors (CCRs) are characterized by their ability to bind to CC chemokines (also referred to as β-chemokines). The CC chemokines are characterized by a conserved cysteine motif, in which the first two cysteines are juxtaposed. The CCR family includes both specific and non-specific receptors. For example, CCR1 is known to bind macrophage inflammation protein-1a (MIP-1a), RANTES (regulation on activation normal T cell expressed and secreted), and monocyte chemoattractant protein-3 (MCP-3) (Neote et al., (1993) *Cell* 72:415-425). CCR2 binds MCP-1, MCP-3, and MCP-4 (Myers et al. (1995) *J. Biol. Chem.* 270:5786-5792; Garcia-Zepeda et al. (1996) *J. Immunol.* 157:5613), whereas CCR3 recognizes eotaxin and MCP-4 (Kitaura et al. (1996) *J. Biol. Chem.* 271:7725; Garcia-Zepada et al. (1996) *J. Immunol.* 157:5613). CCR4 is activated by macrophage inflammatory protein-1a (MIP-1a), RANTES, and MCP-1 (Power et al. (1995) *J. Biol. Chem.* 270:19495). CCR5 was found to bind to and be activated by RANTES , MIP-1a, and MIP-1b (Raport et al. (1996) *J. Biol. Chem.* 271: 17161). CCR6 and CCR7 have recently been discovered and specifically bind to liver and activation regulated chemokine (LARC) and EBI1-ligand chemokine (ELC) respectively (Baba et al, 1997; Yoshida et al. 1997). It is known that CCR10 binds MCP-1 and MCP-3 with high affinity.

The language "aromatic moiety" includes groups with aromaticity, e.g. moieties that have at least one aromatic ring. For example, 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heteroaryls" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido),

amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g., tetralin). In a preferred embodiment, the aromatic moiety of the present invention comprises two aromatic rings, e.g. pyridyl or pyrimidyl rings, e.g. two pyrimidyl rings connected with an ether linkage.

The language "G-protein coupled heptahelical receptor pocket" refers to a region of the GPCR which is capable of interacting with, e.g. binding to, the GPCR pocket binding moiety. Not wishing to be bound by theory, it is believed that the GPCR pocket may be a cavity in the GPCR lined with hydrophobic amino acid residues.

The language "G-protein coupled heptahelical receptor pocket interacting moiety" refers to a group which interacts with a GPCR pocket. The group may be substituted or unsubstituted aromatic, alkyl, alkenyl, cycloalkyl, etc. The interaction between the GPCR pocket interacting moiety and the pocket includes any interaction which allows the compound to perform its intended function, e.g., the interaction is hydrophobic, ionic, covalent, or combinations thereof.

In one aspect of the invention, the GPCR binding compound modulates the recruitment of at least one inflammatory cell type in a subject. The ability of a GPCR binding compound to modulate the recruitment of at least one inflammatory cell type can be measured or observed using art-recognized techniques or assays. Examples of such assays are the Murine Inflammatory Cell Recruitment Assay (referred to herein as the MICR Assay) and the Cell Based Inflammatory Recruitment Assay (referred to herein as the CIBR Assay) as described in Examples 4 and 5, respectively.

The term "subject" includes any animal which expresses GPCRs, for example, mammals e.g., mice, rats, cows, sheep, pigs, horses, monkeys, dogs, cats and, preferably, humans.

The language "inflammatory cell type" includes cell types associated with a chemokine mediated disorder characterized by inflammation. Examples of these cell types include, but are not limited to, leukocytes, e.g., eosinophils, neutrophils, basophils, fibroblasts, monocytes, T lymphocytes, and macrophages. Leukocytes are white blood

cells which are involved in nonspecific resistance against pathogenic microorganisms and inflammatory response. Monocytes are particularly important in the nonspecific immune response, while lymphocytes are especially important in the specific immune response. Neutrophils are the most abundant phagocytic cells in blood and are continuously produced in circulating blood, affording protection against the entry of foreign materials. These leukocytes exhibit chemotaxis and are attracted to foreign substances, including invading microorganisms, which they engulf and digest along with particulate matter. Eosinophils are leukocytes which react with the acidic dye eosin. Basophils are leukocytes which stain with basic dyes. Macrophages are large ameboid mononuclear phagocytic cells.

The present invention also pertains to a GPCR binding compound which is an antagonist of a G-protein coupled heptahelical receptor, e.g., a β-chemokine receptor. In one embodiment, the IC_{50} of the GPCR binding compound to the GPCR is about 10 μM or less, e.g., about 5 μM or less, e.g., about 1 μM or less, e.g., about 50 nM or less.

The term "antagonist" includes compounds which bind to the GPCR such that the binding of a second compound to the GPCR is modulated.

The ability of a compound to bind to a GPCR can be determined through using art-recognized techniques and assays. Examples of such assays include the Time Resolved Fluorescence assay (herein referred to as the TRF assay) and the Direct Binding Assay (herein referred to as the DB Assay), described in Examples 2 and 3, respectively. The TRF assay determines the binding affinity of a compound to a receptor by over expressing the receptor in a culture of cells. The TRF assay determines the binding affinity of a compound to a receptor using a cell line engineered to overexpress a GPCR, e.g. CCR10. The cells are exposed simultaneously to the test compound and a fluorescently labeled ligand specific for the receptor. After a predetermined amount of time, excess ligand and test compound is removed. The amount of fluorescence is measured and the percent inhibition of binding of the ligand is calculated. By repeating this experiment at multiple concentrations of test compound, it is possible to generate a dose-response curve from which an IC_{50} can be determined.

In another aspect, the invention pertains to a method for treating a chemokine mediated disorder, (e.g., a neurological disorder, an immunological disorder, a disorder characterized by inflammation, or a disorder characterized by unwanted cellular proliferation) in a subject. The method includes administering an effective amount of a G-protein coupled heptahelical receptor binding compound to a subject. For example, the disorder may be treated through modulation of a β-chemokine receptor, e.g., CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, or CCR10. Examples of preferred disorders include AIDS, multiple sclerosis, asthma, cancer, and lupus. The disorder may also be characterized by abnormal cellular signal transduction, or amounts of chemokine stimulated chemotaxis.

The term "administering" includes routes of administration which allow the GPCR binding compound to perform its intended function, e.g. interacting with GPCRs and/or treating a chemokine mediated disorder. Examples of routes of administration which can be used include parental injection (e.g., subcutaneous, intravenous, and intramuscular), intraperitoneal injection, oral, inhalation, and transdermal. The injection can be bolus injections or can be continuous infusion. Depending on the route of administration, the GPCR binding compound can be coated with or disposed in a selected material to protect it from natural conditions which may detrimentally effect its ability to perform its intended function. The GPCR binding compound can be administered alone or with a pharmaceutically acceptable carrier. Further, the GPCR binding compound can be administered as a mixture of GPCR binding compounds, which also can be coadministered with a pharmaceutically acceptable carrier. The GPCR binding compound can be administered prior to the onset of a chemokine mediated disorder, or after the onset of a chemokine mediated disorder. The GPCR binding compound also can be administered as a prodrug which is converted to another form *in vivo*.

The term "treatment" includes the diminishment or alleviation of at least one symptom associated or caused by the disorder being treated. For example, treatment can be diminishment of several symptoms of a disorder or complete eradication of a disorder.

The language "chemokine mediated disorder" includes a disorder characterized by the participation of chemokines or association with chemokines. The language also includes disorders characterized by aberrant chemokine expression. Chemokines have a wide variety of functions. They are able to elicit chemotactic migration of distinct cell types, such as monocytes, neutrophils, T lymphocytes, basophils and fibroblasts. Many chemokines have proinflammatory activity and are involved in multiple steps during an inflammatory reaction. These activities include stimulation of histamine release, lysosomal enzyme and leukotriene release, increased adherence of target immune cells to endolethial cells, enhanced binding of complement proteins, induced expression of granulocyte adhesion molecules and complement receptors, and respiratory burst. In addition to their involvement in inflammation, certain chemokines have been shown to exhibit other activities. For example, macrophage inflammatory protein -1 (MIP-1) is able to suppress hematopoietic stem cell proliferation, platelet factor-4 is a potent inhibitor of endolethial cell growth, interleukin-8 (IL-8) promotes proliferation of keratinocytes, and GRO is an autocrine growth factor for myeloma cells. Chemokines have been proposed to participate in a number of physiological and disease conditions, including, for example, lymphocyte trafficking, wound healing, hemapoietic regulation and immunological disorders such as asthma and arthritis.

The language "chemokine mediated disorder characterized by inflammation" includes a disorder having inflammation as at least one of its symptoms. Examples of such disorders include anaphylaxis, systemic necrotizing vasculitis, systemic lupus erthyematosus, serum sickness syndromes, psoriasis, rheumatoid arthritis, adult respiratory distress syndrome (ARDS), allergic rhinitis, atopic dermatitis, asthma and other allergic responses, and reperfusion injury occurring after periods of ischemia such as in myocardial infarction or shock. Preferably, the disorder is asthma.

Other groups of possible chemokine mediated disorders include neurological related disorders, immunological related disorders and disorders characterized by unwanted cellular proliferation, e.g. cancer.

The language "neurological related disorders" includes disorders of the nervous system, including, but not limited to those involving the brain, the central and peripheral nervous system, and the interfaces between muscles and the nerves. Some examples of

neurological related disorders include Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, and Jakob-Creutzfieldt disease. "Neurological related disorders" also includes neurological disorders associated with inflammation, e.g. stroke, traumatic injury to the brain, traumatic injury to the spinal cord, spinal crush, and central and peripheral nervous system trauma.

The language "immunological related disorder" includes both organ-specific and systemic immunological disorders. Some examples of immunological disorders include immune thyroiditis, hyperthyroidism, type I diabetes mellitus, insulin related diabetes, Addison's disease, autoimmune oophoritis, autoimmune orchitis, AIDS, autoimmune hemolytic anemia, paroxysmal cold hemoglobinuria, autoimmune thrombocytopenia, autoimmune neutropenia, pernicious anemia, autoimmune coagulopathies, myasthenia gravis, multiple sclerosis, experimental allergic encephalomyelitis, pemphigus and other bullous diseases, rheumatic carditis, Goodpasture's syndrome, postcardiotomy syndrome, systemic lupus erythematosus, rheumatoid arthritis, keratitis, parotitis, polymositis, dermatomyositis, and scleroderma. Preferably, the immunological disorder is AIDS, multiple sclerosis, rheumatoid arthritis, or lupus.

In another embodiment, the invention pertains to a pharmaceutical preparation comprised of an effective amount of a G-protein coupled heptahelical receptor binding compound and a pharmaceutically acceptable carrier. In a preferred aspect, the effective amount is an effective amount to treat a β-chemokine mediated disorder, e.g., asthma.

The language "pharmaceutically acceptable carrier" includes substances capable of being coadministered with the GPCR binding compound(s), and which allows both to perform their intended function, e.g., treating a chemokine mediated disorder or preventing a chemokine mediated disorder. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. The use of such media for pharmaceutically active substances are well known in the art. Any other conventional carrier suitable for use with the GPCR binding compound also fall within the scope of the present invention.

Furthermore, the language "pharmaceutically acceptable carrier" is art recognized and includes a pharmaceutically acceptable material, composition or vehicle, suitable for administering compounds of the present invention to mammals. The carriers include liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, α-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium

carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluent commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert dilutents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with

a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating

materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical composition.

As set out above, certain embodiments of the present compounds can contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" is art recognized and includes relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, *e.g.*, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances includes relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such

as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the compounds of the present invention. These esters can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters *via* treatment with an alcohol in the presence of a catalyst. Hydroxyls can be converted into esters *via* treatment with an esterifying agent such as alkanoyl halides. The term also includes lower hydrocarbon groups capable of being solvated under physiological conditions, *e.g.*, alkyl esters, methyl, ethyl and propyl esters. (See, for example, Berge et al., *supra.*) A preferred ester group is an acetomethoxy ester group.

The language "effective amount" of the compound is that amount necessary or sufficient to treat or prevent a chemokine mediated disorder, e.g. prevent the various morphological and somatic symptoms of a chemokine mediated disorder. The effective amount can vary depending on such factors as the size and weight of the subject, the type of illness, or the particular GPCR binding compound. For example, the choice of the GPCR binding compound can affect what constitutes an "effective amount". One of ordinary skill in the art would be able to study the aforementioned factors and make the determination regarding the effective amount of the GPCR binding compound without undue experimentation. An *in vivo* assay as described in Example 5 below or an assay similar thereto (e.g., differing in choice of cell line or type of illness) also can be used to determine an "effective amount" of a GPCR binding compound. The ordinarily skilled artisan would select an appropriate amount of a GPCR binding compound for use in the aforementioned *in vivo* assay.

The regimen of administration can affect what constitutes an effective amount. The GPCR binding compound can be administered to the subject either prior to or after the onset of a chemokine mediated disorder. Further, several divided dosages, as well as staggered dosages, can be administered daily or sequentially, or the dose can be continuously infused, or can be a bolus injection. Further, the dosages of the GPCR binding compound(s) can be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

In yet another aspect, the invention features a protein coupled heptahelical receptor binding compound comprising a G-protein coupled heptahelical receptor binding compound packaged with instructions for using said compound for treating a β-chemokine mediated disorder.

The invention also features a method of using a G-protein coupled heptahelical receptor binding compound to modulate the binding of a second compound to a G-protein coupled heptahelical receptor.

In another aspect, the invention pertains to a compound represented by the formula:

$$A\text{-}L_1\text{-}B\text{-}L_2\text{-}E \qquad (II)$$

where A is selected from the group consisting of branched and straight chain alkyl, aryl, alkenyl, alkynyl, and heteroaryl moieties optionally substituted by NR'R", CN, NO_2, F, Cl, Br, I, CF_3, CCl_3, CHF_2, $CHCl_2$, CONR'R", S(O)NR'R", CHO, OCF_3, $OCCl_3$, SCF_3, $SCCl_3$, COR', CO_2R', and OR' and wherein R' and R" are each independently hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, or optionally substituted aryl;

L_1 is a linker moiety selected from the group consisting of a bond, O, S, CHOH, CHSH, $CHNH_2$, CHNHR, CHNRR', NH, NR, $(CH_2)_n$, $O(CH_2)_n$, and $(CH_2)_nO(CH_2)_n$, an optionally substituted ring moiety of 4 to 7 atoms containing up to three heteroatoms, a chain of 1 to 5 atoms optionally substituted by C_1-C_6 alkyl. halogens, wherein n is either 0, 1, 2, or 3, and R and R' are each independently substituted or unsubstituted C_1-C_6 branched or straight chain alkyl, C_1-C_6 branched or straight chain alkenyl, aryl, C_4-C_7 ring, optionally substituted with up to three heteroatoms;

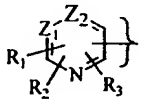
B is an aromatic moiety containing from 0 to 3 heteroatoms and containing 5 to 7 members optionally substituted by NR'R", cyano, nitro, halogen, CF_3, CHF_2, CONR'R", S(O)NR'R", CHO, OCF_3, SCF_3, COR', CO_2R', OR' where R' and R" are each independently hydrogen, halogen, C1-C6 alkyl, optionally substituted aryl or optionally substituted aryl;

L_2 is a second linking moiety selected from the group consisting of a bond, $CH_2C=O$, NHC=O, OC=O, C=O, $CH_2NHC=O$, CHOH, $(CH_2)_n$, O, NH, $O(CH_2)_n$, $NH(CH_2)_n$, CH_2CHOH and NRC=O; and

E is a G-protein coupled heptahelical receptor pocket interacting moiety.

In one aspect, L_1 is S, NH, CH_2, or O. In another aspect, L_2 is NHC=O.

In one embodiment, A is represented by the following formula:

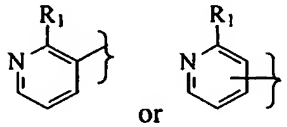


wherein

Z_1 and Z_2 each independently represent N or C;

R_1, R_2, and R_3 are independently selected from the group comprised of hydrogen, C_1-C_{12} branched or straight chain alkyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl.

In one embodiment, both Z_1 and Z_2 are carbon, R_1 is alkyl (e.g., methyl), halogen (e.g., bromine, chlorine or fluorine), or alkoxy and the L_1 linker is located in the meta position. For example, A may be represented by the following formula:



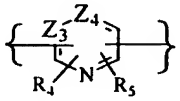
or .

In another embodiment, R_1 is carbonyl (e.g., a ketone, an aldehyde, an ester, or an amide.). Furthermore, R_1 may be substituted with a cyclic moiety such as piperazine, furan, or phenyl.

In yet another embodiment, A is substituted or unsubstituted phenyl. Examples of substituents includes substituted or unsubstituted alkyl (e.g., methyl), alkenyl, aryl and heteroaryl moieties. Furthermore, A may be substituted with halogens (e.g., chlorine).

In one embodiment, L_1 is O.

In another aspect, the invention features a compound wherein B is represented by the following formula:

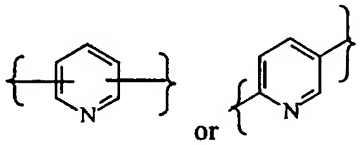


wherein

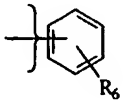
Z_3 and Z_4 each independently represent N or C;

R_4 and R_5 are independently selected from the group comprised of hydrogen, C_1-C_6 branched or straight chain alkyl, alkenyl, alkynyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl. In one embodiment, R_4 is alkyl and R_5 is hydrogen.

In one embodiment, B is a substituted or unsubstituted pyridyl or pyrimidyl moiety, wherein B may be represented by the following formula:

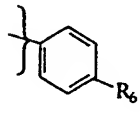


In an aspect, the invention features a compound wherein E is represented by the formula below:



wherein R_6 is an electron withdrawing moiety and the aryl ring is additionally optionally substituted with zero to four halogen atoms. Preferably, E is substituted with at least one fluorine atom, e.g. two or more fluorine atoms. For example, R_6 may be alkyl, alkoxy, haloalkyl, nitro, halo, alkylamino, hydroxyalkyl, or carboxyl.

In an embodiment, E is a para substituted aryl moiety represented by the formula below:

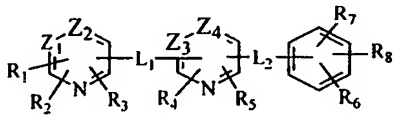


In a further embodiment, R_6 is a halogenated alkyl moiety, e.g. a fluorinated alkyl moiety, e.g. a trifluoromethyl or pentafluoroethyl. Other examples of R_6 include substituted or unsubstituted alkoxy moieties (e.g., methoxy, trifluoromethoxy) or thioether moieties. R_6 can be alkenyl or alkynyl (e.g., ethenyl).

Furthermore, in another embodiment E is heterocyclic, e.g., substituted or unsubstituted furanyl, imidazolyl, benzothiophenyl, benzylfuranyl, quinolinyl, isoquinolinyl, benzodioxazolyl, benzoxazolyl, benzothiazolyl, benzylimidazolyl, thiazolyl, isothiaozolyl, oxazolyl, benzylthiazolyl, isooxazolyl, methylenedioxyphenyl, indolyl, thienyl, pyrimidyl, pyrazinyl, purinyl, or deazapurinyl.

In yet another embodiment, E is branched or straight chain alkenyl or alkynyl. Examples include ethynyl trimethyl silane and alkenes (e.g., dienes, trienes).

In another aspect, the invention features a compound represented by the formula below:



(III)

wherein

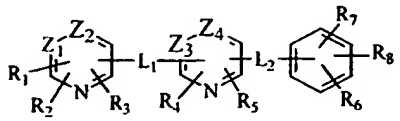
Z_1, Z_2, Z_3, and Z_4 are each independently N or C;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, C_1-C_6 branched or straight chain alkyl, alkenyl, alkynyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl;

L_1 is O, S, NH, NR_7, $(CHR_7)_n$, CO, CR_7OH, $O(CHR_7)_n$, and $(CHR_7)_nO(CHR_7)_n$ wherein n is either 1,2, or 3;

L_2 is a second linking moiety selected from the group consisting of a bond, $CH_2C{=}O$, $NHC{=}O$, $OC{=}O$, $C{=}O$, $CH_2NHC{=}O$, $NHC{=}OCH_2$, CHOH, $(CH_2)_n$, O, NH, $O(CH_2)_m$, $NH(CH_2)_m$, CH_2CHOH and $NRC{=}O$, wherein m is 0, 1, 2, or 3.

Furthermore, the compound may be represented by the formula below:



(IV)

wherein

Z_1, Z_2, Z_3, and Z_4 are each independently N or C;

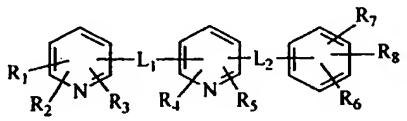
R_1, R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, C_1-C_6 branched or straight chain alkyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl;

L_1 is O, S, NH, NR_7, $(CH_2)_n$, CO, CHOH, $O(CH_2)_n$, and $(CH_2)_nO(CH_2)_n$ wherein n is either 1,2, or 3 and R_7 is C_1-C_6 branched or straight chain alkyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl;

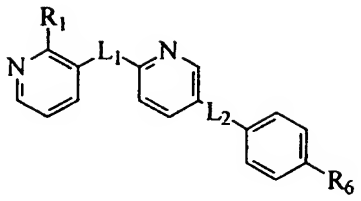
L_2 is a second linking moiety selected from the group consisting of a bond, $CH_2C=O$, NHC=O, OC=O, C=O, $CH_2NHC=O$, CHOH, $(CH_2)_n$, O, NH, $O(CH_2)_n$, $NH(CH_2)_n$, CH_2CHOH and NRC=O.

In one embodiment, Z_1 and Z_2 are both carbon. In another, R_1 is methyl, and R_2 and R_3 are hydrogen. In another aspect, L_1 is O. In an yet another embodiment, R_4 and R_5 are both hydrogen. In yet another embodiment, L_2 is NHC=O. In a preferred aspect, R_6 is a halogenated alkyl moiety, e.g. a fluorinated alkyl moiety, e.g. a trifluoromethyl or pentafluoroethyl moiety. In another aspect, R_7 and R_8 are each independently fluorine or hydrogen.

In yet another aspect, the invention can be represented by the structure below:

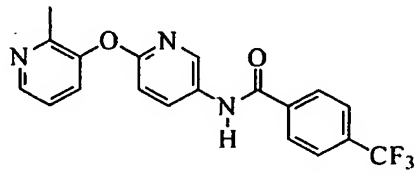


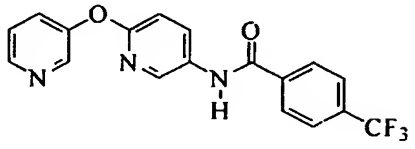
The invention also features compounds of the formula:

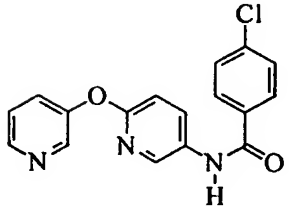


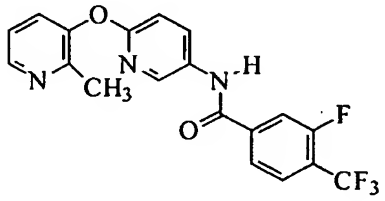
In one embodiment, L_1 is O and L_2 is NHCO. In a further embodiment, L_1 is O, L_2 is NHC=O, and R_6 is a halogen, a halogenated alkyl group, e.g. trifluoromethyl or pentafluoroethyl), or an alkoxy group, e.g. a halogenated alkoxy group, e.g. a trifluoromethoxy group. Furthermore, R_6 also can be ethenyl or a thioether moiety (e.g. $-S-CF_3$).

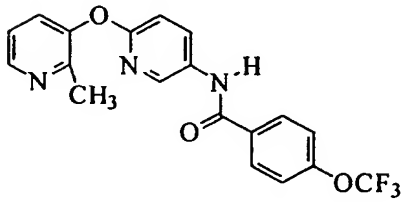
The invention also features binding compounds represented by the following structures:

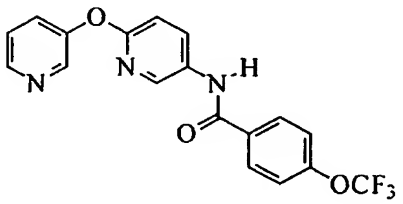


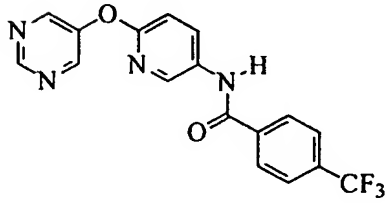


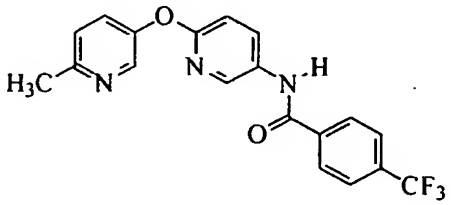


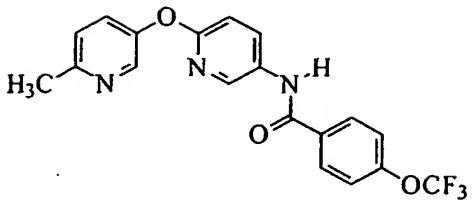


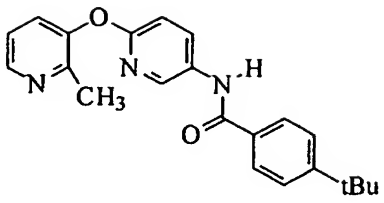


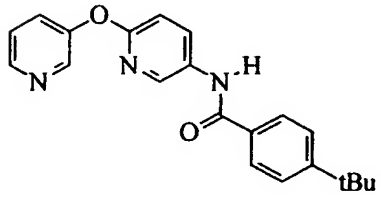


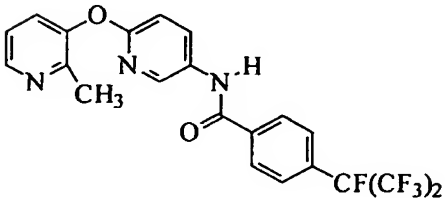


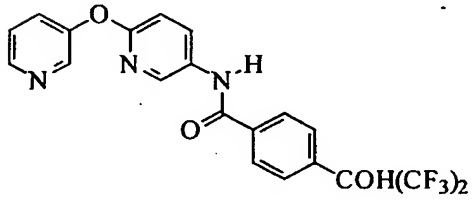


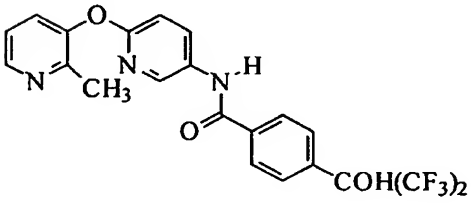


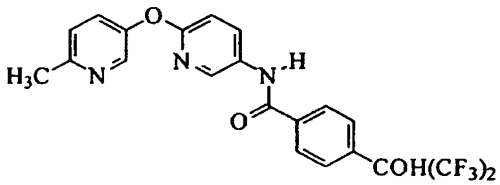


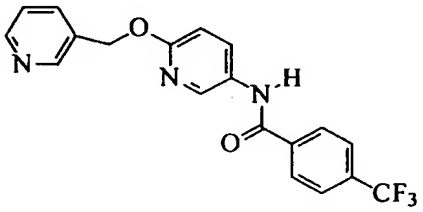


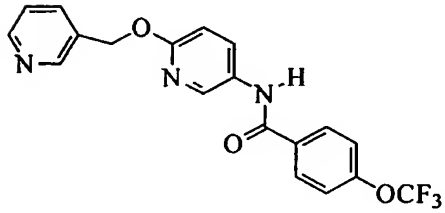


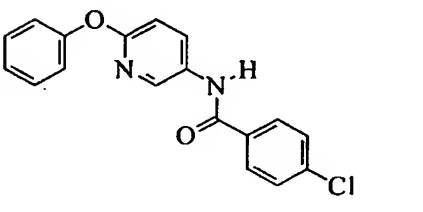


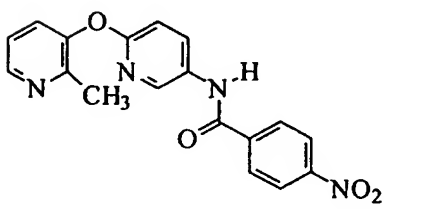


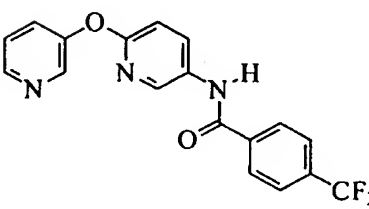


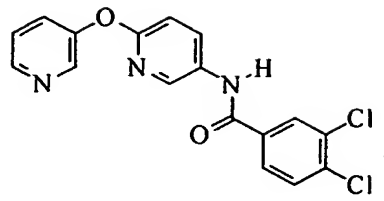
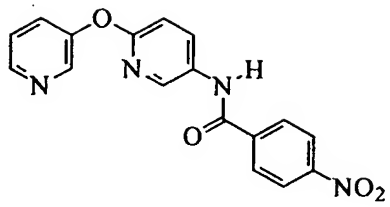
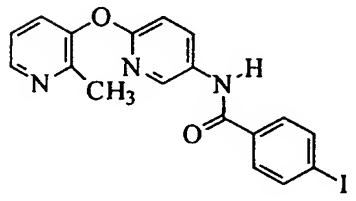
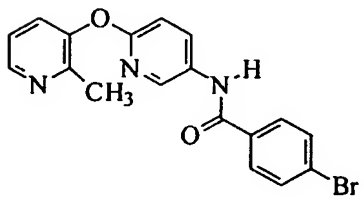
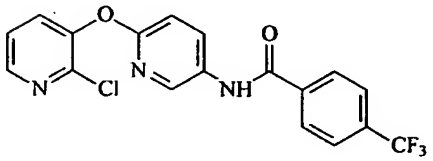
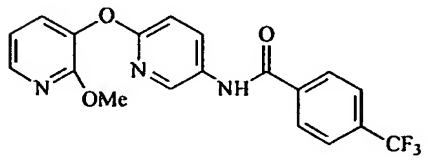
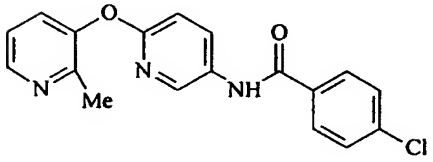
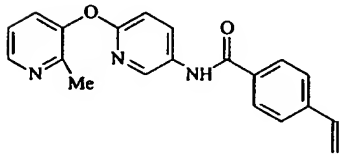
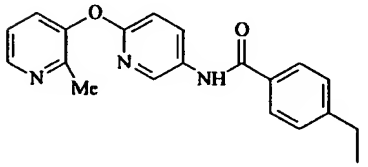
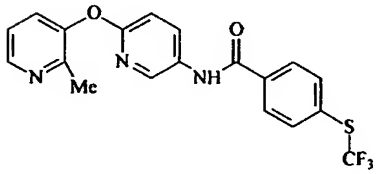
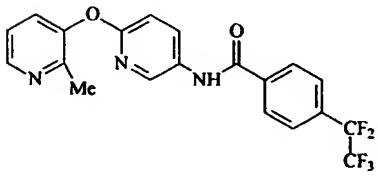
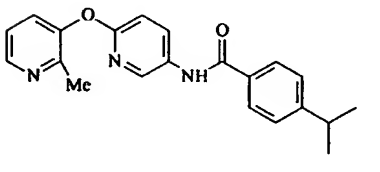










The term "alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. The term alkyl further includes alkyl groups, which can further include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone, e.g., oxygen, nitrogen, sulfur or phosphorous atoms. In preferred embodiments, a straight chain or branched chain alkyl has 10 or fewer carbon atoms in its backbone (e.g., C_1-

C_{10} for straight chain, C_3-C_{10} for branched chain), and more preferably 6 or fewer. Likewise, preferred cycloalkyls have from 4-7 carbon atoms in their ring structure, and more preferably have 5 or 6 carbons in the ring structure.

Moreover, the term alkyl includes both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, silyl, trialkylsilyl, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "alkylaryl" moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)).

The term "aryl" includes aryl groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heteroaryls" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido),

amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g., tetralin).

The terms "alkenyl" and "alkynyl" include unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond, respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to three carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

The terms "alkoxyalkyl", "polyaminoalkyl" and "thioalkoxyalkyl" include alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, e.g., oxygen, nitrogen or sulfur atoms.

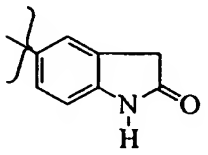
The terms "polycyclyl" or "polycyclic radical" refer to two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (e.g., all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis.

In one embodiment, the present invention includes GPCR binding compounds and/or methods of using the same which are encompassed by the formulae set forth herein and which are not described in Brombridge, et al., *J. Med Chem.* (1997) 40:3494, U.S. 3,499,898, EP358 118, EP 344 634, and/or WO 96/23783. The contents of each of which are expressly incorporated herein by reference.

In another embodiment, E or the G-protein coupled heptahelical pocket interacting moiety is not:



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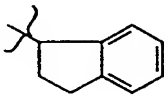
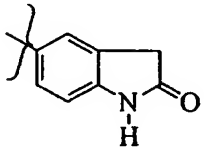

, and/or unsubstituted phenyl.

In another embodiment, L_1 is O, L_2 is NHCO, and E or the G-protein coupled heptahelical pocket interacting moiety is not:



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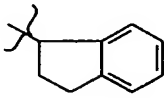
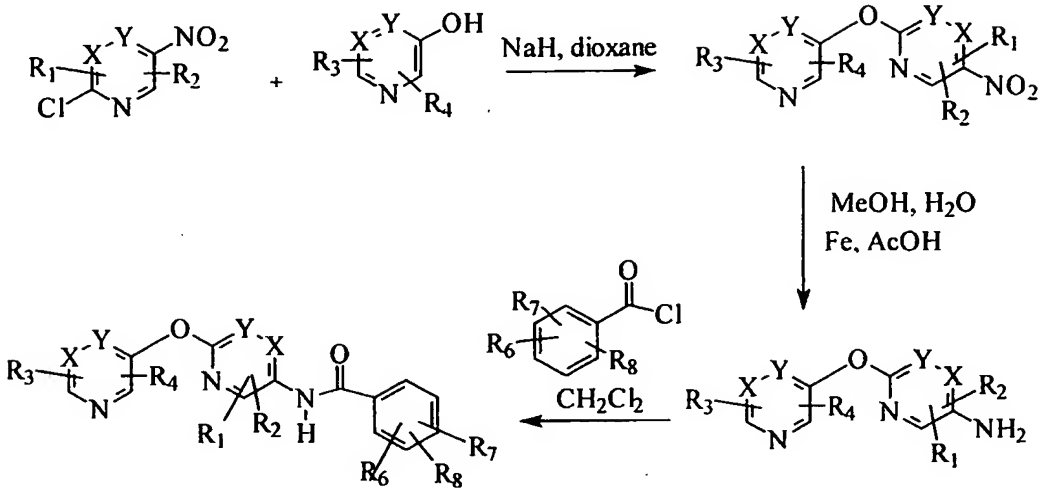

, and/or unsubstituted phenyl.

In another embodiment, E or the G-protein coupled heptahelical pocket interacting moiety is a phenyl group having at least one substituent. In yet another embodiment, E is a phenyl group having at least one substituent in the para position. In a further embodiment, E is a phenyl group having at least two substituents.

The compounds of the present invention can be synthesized using standard methods of chemical synthesis and/or can be synthesized using schemes described herein. Synthesis of specific compounds is discussed in detail in the Example sections below. An example of a general synthesis is outlined in the scheme below:

Scheme 1: General Synthesis of Test Compounds



Hydroxypyridine (or other hydroxyaryl precursor) is dissolved in dioxane and 1.5 equivalents of 95% sodium hydride is added. The mixture is stirred at room temperature for 20 minutes and 1 equivalent of 2-chloro-5-nitropyridine (or other α-chloro-heterocycle) is then added. The mixture is subsequently brought to reflux for 3 hours and cooled. The reaction mixture is then quenched by addition of saturated ammonium chloride solution. Silica gel is added to the solution and the mixture is rotovapped to dryness. The product is eluted from the silica gel and flash chromatographed using a mixture of ethyl acetate/hexane.

The nitro group is reduced by dissolving the nitropyridine in 1:1 methanol:water. Acetic acid and iron powder is then added and the mixture is brought to reflux for 3 hours. After cooling, the iron is precipitated by addition of 20% NaOH and subsequently filtered through Celite. The methanol is removed by rotary evaporation and the remaining aqueous mixture is extracted with methylene chloride. The organic layer is dried and the solvent removed by rotary evaporation to give product.

The dipyridyl ether is then dissolved in methylene chloride followed the addition of polymer bound morpholine (Booth, *et al.*, *J. Am. Chem. Soc.*, *119*, 1997, 4882-4886). 1.3 equivalents of acid chloride is added and the mixture shaken overnight. The excess acid chloride is scavenged using polymer bound tris-2-aminoethylamine. Filtration

followed by flash chromatography of the filtrate using ethyl acetate:hexane gives the product.

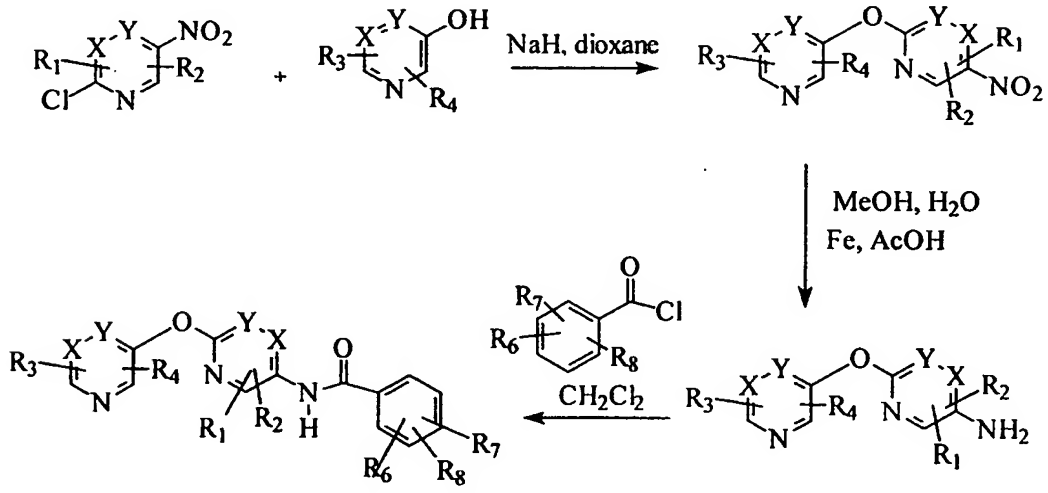
Further examples of syntheses of compounds of the invention are included in the Example section.

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. The contents of all references, pending patent applications and published patent applications, cited throughout this application are hereby incorporated by reference. It should be understood that the animal models used throughout the examples are accepted animal models and that the demonstration of efficacy in these animal models is predictive of efficacy in humans.

EXAMPLE 1: Synthesis of GPCR Binding Compounds

A. General Procedures

Many GPCR binding compounds of the invention were made by the general reaction scheme, outlined below:



I: Nucleophilic displacement of 2-chloroheterocycles

Method A:

50 mmol of hydroxypyridine (or other hydroxyaryl precursor) is dissolved in 500 mL of dioxane. 1.5 equivalents of 95% sodium hydride is added and the mixture stirred at room temperature for 20 minutes. 1 equivalent of 2-chloro-5-nitropyridine (or other α-chloro-heterocycle) is added and the mixture is brought to reflux for 3 hours. After cooling, the reaction mixture is quenched by addition of 2 mL of saturated ammonium chloride solution. 10 g of silica gel is added to the solution and the mixture is rotovapped to dryness. The product is eluted from the silica gel and flash chromatographed using a mixture of ethyl acetate/hexane.

Method B:

50 mmol of hydroxypyridine is dissolved in 150 mL of dry DMF. The mixture is cooled to 0 C and 1.5 equivalents of sodium hydride added. The mixture is allowed to warm to room temperature over 20 minutes, followed by addition of the α–chloro heterocycle. The reaction mixture is stirred at room temperature for 16 hours, and then partitioned between ethyl acetate/water in a separatory funnel. The organic layer is dried and the solvent removed to give product which is typically > 90% pure.

II: Reduction of Nitro group

Method A:

40 mmol of nitropyridine is dissolved in 200 mL of methanol followed by the addition of 200 mL of water. Acetic acid (8.3 mL) and iron powder (17 g) is added and the mixture is brought to reflux for 3 hours. After cooling, the iron is precipitated by addition of 25 mL of 20% NaOH and the mixture filtered through Celite. Removal of the methanol by rotary evaporation is followed by extraction of the remaining aqueous mixture with methylene chloride. The organic layer is dried and the solvent removed by rotary evaporation to give product.

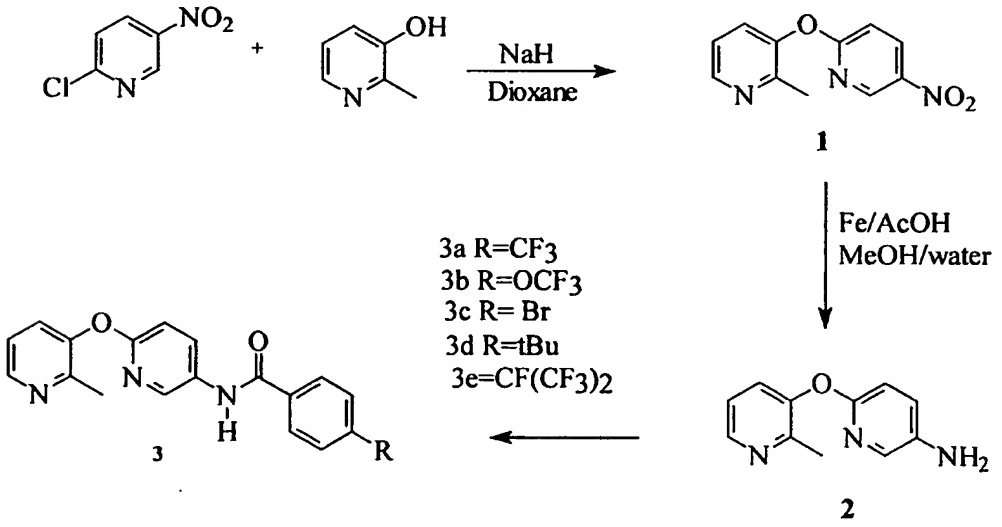
Method B:

40 mmol of nitropyridine is dissolved in 250 mL of ethanol and treated with a mixture of tin(II) chloride (30 g) in conc. HCl (65 mL). The mixture is heated to 50 C for 1 hour. After cooling, the mixture is basified with 40% NaOH and the product extracted into ethyl acetate. The solution is dried over sodium sulfate and the solvent removed to give product.

III: Acylation of the amino-heterocycle

1 mmol of the dipyridyl ether is dissolved in 12 mL of methylene chloride followed by the addition of 0.4 g of polymer bound morpholine (loading 4 mmol/g) (Booth, *et al. J. Am. Chem. Soc.*, *119*, 1997, 4882-4886). 1.3 equivalents of acid chloride is added and the mixture shaken overnight. The excess acid chloride is scavenged using polymer bound tris-2-aminoethylamine. Filtration followed by flash chromatography of the filtrate using ethyl acetate:hexane gives the product.

B. Synthesis of the GPCR Binding Compounds



(1) 2-methyl-3-hydroxy pyridine (5 g, 46 mmol) and 2-chloro-5-nitro pyridine were suspended in 500 mL of dioxane. Sodium hydride (1.7 g of 95%, 1.5 eq) was added and the mixture refluxed under argon for 3 hours. After cooling, the excess sodium hydride was quenched by addition of 2 mL of saturated ammonium chloride solution and 10 g of silica gel added to the solution. The solvents were removed by rotary evaporation leaving the product adsorbed on silica. Purification was achieved by flash chromatography using 1:1 ethyl acetate/hexanes as eluent. Yield: 9.3 g, 87%. LC-MS (diode array detection at 210-300 nM) showed that the product was > 95% pure and had the expected M.W. of 232 ($M+H^+$). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.98 (d, 1H) δ 8.52 (dd, 1H) δ 8.42 (dd, 1H) δ 7.30 (dd, 1H) δ 7.2 (m, 1H) δ 7.08 (d, 1H) δ 2.38 (s, 3H).

(2) Compound (1) (9.3 g) was suspended in 400 mL of 1/1 methanol/water. Acetic acid (8.3 mL) and powdered iron (17 g) were added and the mixture brought to reflux for 3 hours. After cooling, the iron was precipitated by addition of 25 mL of 20% NaOH. The mixture was filtered through Celite and the filter cake rinsed with methanol. The methanol was removed by rotary evaporation and the product partitioned into methylene chloride in a separatory funnel. Removal of the solvent by rotary evaporation gave 4.1 g (51%)of product. LC-MS showed the product to be > 95% pure and to have the expected M.W. of 202 ($M+H^+$). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.24 (d, 1H) δ 7.60 (d, 1H) δ 7.24 (dd, 1H) δ 7.08 (m, 2H) δ 6.88 (d, 1H) δ 3.52 (br s, 2H) δ 2.38 (s, 3H).

(3a)Compound (2) (202 mg, 1 mmol) was dissolved in 10 mL of methylene chloride in a 20 mL scintillation vial. Polystyrene bound morpholine (0.4 g, loading 4 mmol/g) was added followed by 1.3 mmol of 4-trifluoromethylbenzoyl chloride. The mixture was shaken at room temperature for 12 hours followed by the addition if 0.5 g of polystyrene supported tris-2-aminoethylamine (loading 4 mmol/g). The mixture was shaken for an additional 12 hours and filtered through a polystyrene frit. The reaction mixture was applied to a 90 g cartridge of silica gel and the product eluted with ethyl acetate.

Removal of the solvent gave 248 mg of product. LC-MS indicated a purity > 97%and the expected M.W. of 374 ($M+H^+$). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.79 (s, 1H) δ 8.19 (m, 2H) δ 7.99 (d, 1H) δ 7.84 (d, 2H) δ 7.53 (d, 2H) δ 7.21 (d, 1H) δ 7.06 (m, 1H) δ 6.81 (d, 1H) δ 2.20 (s, 3H). Anal. Calcd for $C_{19}H_{14}F3N_3O_2$: C, 61.13 H, 3.78 N, 11.26 Found: C, 61.27 H, 3.76 N, 11.17.

A number of similar analogs were made from intermediate (**2**) by acylation with other substituted benzoyl chlorides:

(**3b**) LC-MS showed the product to be > 95% pure and to have the expected M.W. of 390 ($M+H^+$). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm) δ 9.02 (s, 1H) δ 8.18 (d, H) δ 8.06 (d, 1H) δ 7.97 (d, 1H) δ 7.8 (m, 2H) δ 7.21 (d, 1H) δ 7.05 (m, 3H) δ 6.8 (d, 1H) δ 2.20 (s, 3H).

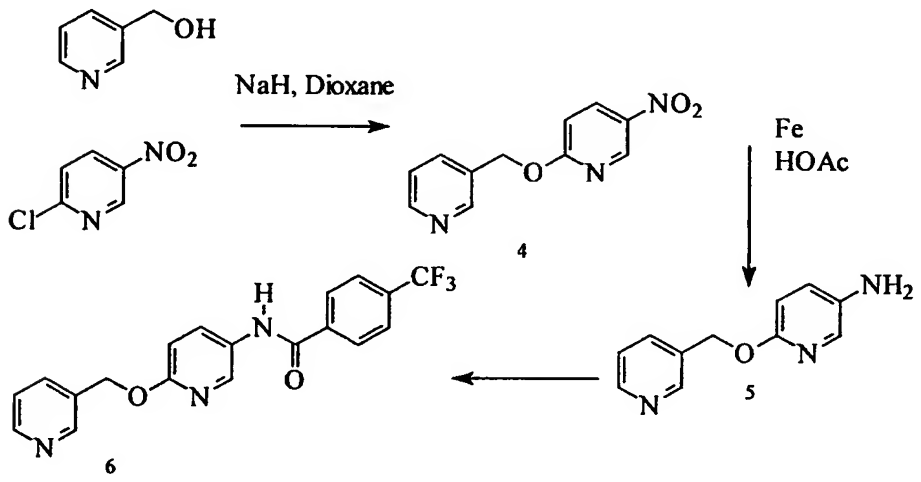
(**3c**) LC-MS showed the product to be > 95% pure and to have the expected M.W. of 385 ($M+H^+$). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 9.18 (s, 1H) δ 8.24 (d, 1H) δ 8.18 (d, 1H) δ 8.04 (s, 1H) δ 7.66 (d, 2H) δ 7.45 (d, 2H) δ 7.27 (d, 1H) δ 7.08 (m, 1H) δ 6.80 (d, 1H) δ 2.21 (s, 3H).

(**3d**) LC-MS showed the product to be > 95% pure and to have the expected M.W. of 362 ($M+H^+$). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 9.18 (d, 1H) δ 8.22 (d, 1H) δ 8.19 (d, 1H) δ 8.17 (d, 1H) δ 7.78 (d, 2H) δ 7.36 (d, 2H) δ 7.26 (d, 1H) δ δ 7.05 M, 1H) δ 6.8 (d, 1H) δ 2.22 (s, 3H) δ 1.20 (s, 9H).

(**3e**) 4-(perfluoroisopropyl)benzoyl chloride was made as follows: 4-iodo benzoic acid (5 g, 20.2 mmol) was dissolved in DMF along with perfluoroisopropyl iodide (6 g, 20.3 mmol). Copper powder (6.35 g) and DMF (25 mL) were added and the mixture was heated at 140 C in a sealed tube for 8 hours. After cooling, the reaction mixture was partitioned between diethyl ether and 1 N HCl. The organic layer was separated and dried and the resulting solid chromatographed on silica (ethyl acetate as eluent) to give a

mixture of compounds containing the desired product (by LC-MS.) The crude mixture was treated with oxalyl chloride (3 mL) in 25 mL of methylene chloride. After 3 hours of stirring, the solvents were removed by rotary evaporation and residual oxalyl chloride removed by azeotroping with toluene. The crude acid chloride was used to acylate compound (**2**) according to the general procedure given above. The product was purified by preparative TLC (5:4:1 methylene chloride:ethyl acetate:methanol). LC-MS showed the product to be > 95% pure and to have the expected M.W. of 474 ($M+H^+$) ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.25 (m, 3H) δ 8.19 (s, 1H) δ 8.05 (d, 2H) δ 7.79 (d, 2H) δ 7.4 (d, 1H) δ 7.2 (m, 2H) δ 6.99 (d, 1H) δ 2.2 (s, 3H).

C. Synthesis of "Extended Bridge" GPCR Binding Compounds



(**4**) 3-pyridinol (5g, 45.8 mmol) was dissolved in 500 mL of dioxane. Sodium hydride (1.7 g, 1.5 equivalents) was added and the mixture stirred for 10 minutes. 2-chloro-5-nitro pyridine (7.27 g, 45.8 mmol) was added and the mixture was brought to reflux for 3 hours. After cooling, the excess sodium hydride was quenched with 2 mL of saturated aqueous ammonium chloride and 10 g of silica added to the mixture. Removal of the solvent left the product adsorbed on silica gel. Elution with ethyl acetate: hexane (1:1) combined with flash chromatography gave 5.4 g of product. LC-MS indicated a purity

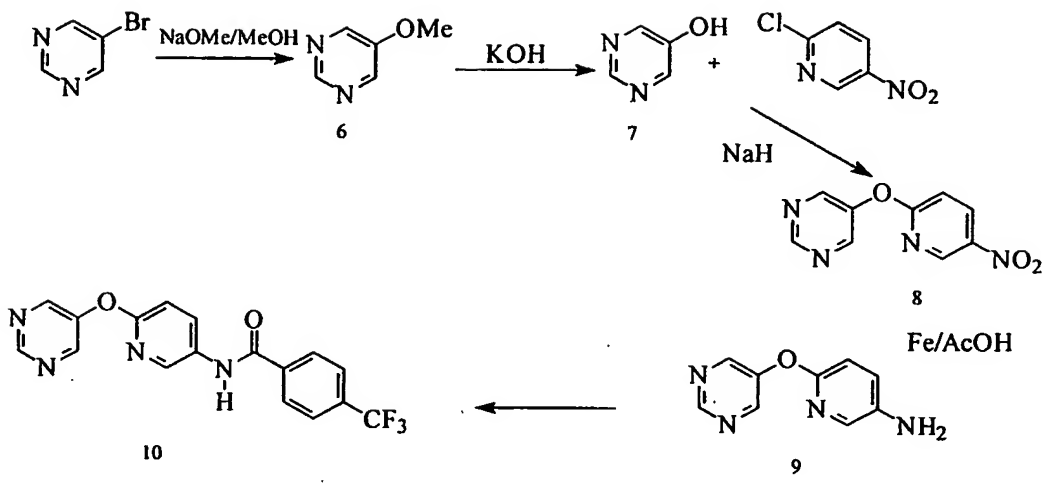
of > 95%. ^{1}H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 9.08 (d, 1H) δ 8.65-8.8 (br d, 2H) δ 8.32 (dd, 1H) δ 7.79 (d, 1H) δ 7.27 (m, 1H) δ 6.81 (d, 1H) δ 5.44 (s, 2H). MW=231. $M+H^+$=232.

(**5**) The product from above (**4**) was dissolved in 45 mL of methanol and 45 mL of water added. Iron powder (3.6 g) and acetic acid (1.72 mL) were added and the mixture brought to reflux for 3 hours. After cooling, the iron was precipitated by addition of 20% NaOH and the mixture filtered through Celite. Removal of the methanol by rotary evaporation was followed by extraction of the product into methylene chloride. Removal of the solvent gave 1.08 g of product (**5**). LC-MS indicated a purity of >95%. ^{1}H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.43 (d, 1H) δ 8.25 (d, 1H) δ 7.58 (d, 1H) δ 7.42 (d, 1H) δ 7.02 (dd, 1H) δ 6.8 (dd, 1H) δ 6.4 (d, 1H) δ 5.1 (s, 2H) δ 3.2 (br s, 2H). MW=201. $M+H^+$=202.

(**6**) Compound (**5**) from above (1 mmol, 202 mg) was dissolved in 12 mL of methylene chloride. 0.4 g of polymer bound morpholine was added, followed by 1.3 mmol of 4-trifluoromethylbenzoyl chloride. The mixture was shaken at room temperature for 12 hours, at which time 0.5 g of polymer bound tris-2-aminoethylamine was added to scavenge excess acid chloride. After an additional 12 hours of shaking, the reaction mixture was filtered through a polystyrene frit and chromatographed on silica using ethyl acetate as eluent. Yield: 340 mg. LC-MS indicated the product was > 95% pure. ^{1}H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.86 (s, 1H) δ 8.55 (d, 1H) δ 8.25, (s, 1H) δ 8.07 (s, 1H) δ 7.99 (m, 3H) δ 7.81 (d, 1H) δ 7.71(d, 2H) δ 7.27 (m, 1H) δ 6.80 (d, 1H) δ 5.39 (s, 2H). MW=373

D. Synthesis of A-ring Pyrimidyl GPCR Binding Compounds

The general procedure is analogous to those described above, the only difference being that the starting material was 5-hydroxypyrimidine rather than 3-hydroxypyridine.



(6) 5-bromo pyrimidine (8 g, 50.6 mmol) was dissolved in 100 mL of methanol containing 3.14 g of sodium methoxide. The mixture was sealed in a glass pressure vessel and heated to 130 C for 3 hours. After cooling, 10 g of silica was added to the mixture and the solvent removed by rotary evaporation. The product was eluted from the silica and flash chromatographed using 1:1 hexanes/ethyl acetate. Yield: 2.5 g LC-MS showed the product was > 95% pure and had the correct M.W. ($M+H^+$= 111.0) ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.82 (s, 1H) δ 8.40 (s, 2H) δ 3.85 (s, 3H).

(7) 5-methoxy pyrimidine (4.0 g, 36.4 mmol) was dissolved in 30 mL of dry ethylene glycol. Powdered KOH (10 g) was added and the mixture refluxed under argon for 3 hours. Excess ethylene glycol was removed by rotary evaporation at 0.5 mm Hg/130 C. The product was extracted from the residue using several portions of boiling dioxane. Removal of the dioxane gave a viscous solution which, upon cooling, deposited white needles of 5-hydroxypyrimidine. LC-MS showed the product to be > 95% pure and of the expected M.W. ($M+H^+$=97). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm): δ 8.51 (s, 1H) δ 8.20 (s, 2H).

(**8**) 5-hydroxy pyrimidine (0.35 g, 3.64 mmol) was dissolved in 15 mL of dry DMF. Sodium hydride (0.14 g, 1.6 eq) was added and the mixture stirred for ½ hour at 0 C. 2-chloro-5-nitro pyridine ((0.867 g, 1.5 eq) was added and the mixture stirred at room temperature for 12 hours. The reaction mixture was partitioned between ethyl acetate and sodium bicarbonate solution and the organic layer separated and mixed with 10 g of silica gel. Removal of the solvent left the product adsorbed on silica. The product was eluted onto a flash column with ethyl acetate and fractions containing product combined and rotovapped to dryness. Yield: 0.7 g. LC-MS showed the product to be > 95% pure but the expected parent ion was not observed. NMR was consistent with the expected product: δ 9.19 (s, 1H) δ 9.02 (s, 1H) δ 8.89 (br s, 2H) δ 8.58 (dd, 1H) δ 7.25 (d, 1H).

(**9**) Nitropyrimidyl-pyridine (**8**) was dissolved in 40 mL of 50% water/methanol containing 0.77 g of acetic acid. 1.54 g of powdered iron was added and the mixture brought to reflux for 3 hours. The iron oxides were precipitated by addition of 2 mL of 20% NaOH . Filtration of the reaction mixture through Celite was followed by removal of the methanol by rotary evaporation. The product was extracted into methylene chloride and the solvent removed by rotary evaporation. Yield: 0.37 g. LC-MS showed the product to be > 90% pure and of the expected M.W. ($M+H^+$=189). ^1H NMR ($CDCl_3$, shifts relative to the solvent peak at 7.24 ppm) δ 8.98 (s, 1H) δ 8.60 (s, 2H) δ 7.60 (d, 1H) δ 7.18 (dd, 1H) δ 6.80 (dd, 1H) δ 3.60 (br s, 2H).

(**10**) pyrimidyl-pyridine (**9**) (189 mg, 1 mmol) was dissolved in 12 mL of methylene chloride along with 0.4 g of polymer bound morpholine. 4-trifluoromethylbenzoyl chloride (270 mg, 1.3 mmol) was added and the mixture shaken at room temperature for 12 hours. Polymer bound tris-2-aminoethylamine (0.5 g) was added and the mixture shaken for an additional 12 hours. Filtration of the reaction mixture followed by flash chromatography using ethyl acetate as eluent gave 230 mg of the expected product. LC-MS showed a purity of > 95% and the expected M.W. ($M+H^+$=361). ^1H NMR ($CDCl_3$,

shifts relative to the solvent peak at 7.24 ppm): δ 9.01 (s, 1H) δ 8.68 (m, 3H) δ 8.35 (dd, 1H) δ 8.22 (d, 1H) δ 8.01 (d, 2H) δ 7.75 (d, 1H) δ 7.20 (d, 1H).

Example 2: Identification of GPCR Binding Compounds which Interact with a GPCR Using a Time Resolved Fluorescence (TRF) Assay

High-Throughput Receptor Binding Screening for Antagonists of CCR10

A. Tissue Culture and Production of CCR10 Cells

CCR10 cells are stable recombinant K293 cells overexpressing the CCR10 receptor. The cells are routinely cultured and passaged in a growth medium composed of DMEM base medium: 10% fetal bovine serum (FBS), 1X Glutamine, and 0.4 mg/ml G418. 1% Pen/Strep is also included in the media when the cells are seeded into the plates on Day 1.

B. Labeling of rhMCP-1 with a Europium Chelate

rhMCP-1 (carrier free) was supplied as a frozen stock solution in PBS at a concentration of 0.65 mg/ml. 250 µg (385 µl) was buffer exchanged on a 2.8 ml column of Sephardex G-25 (fine), equilibrated with 10mM Borax, pH 9. Fractions (250 µl) were collected and 2 µl aliquots were analyzed using a Bradford protein assay. Four fractions eluting immediately after the void volume contained the bulk of the protein and were pooled (approx. 1 ml). 1 mg of Eu-labeling reagent (1.5 µmol) was dissolved in 200 µl of water and 100 µl of this solution was added to the protein pool. The final relative concentration ratios of Eu:MCP-1 was about 26:1. The ratio of Eu:NH_2 was about 3:1.

The mixture was incubated for 20 hours at 4°C and then desalted on a 15 mL column of Sephardex G-25 (fine) equilibrated with 20 mM HEPES (hemi-sodium salt), pH 7.5, and 0.9% NaCl. 0.5 mL fractions were collected and 1 µl aliquots were added to

100 µl of enhancement solution and counted on the VICTOR. The five fractions that were eluted immediately after the void volume showed a peak of Eu containing material and were pooled. 60 µl of the pool was set aside for protein assay. 36 µl of 0.1% BSA (heavy metal free; Wallac) was added as a stabilizer to the remainder. The material was then stored at 4°C. The total volume was about 2.5 ml.

The MCP-1 concentration was determined by a BCA protein assay with BSA as a standard to be 8.5 µM (74 µg/ml).

The Eu concentration of the material was measured on the VICTOR with a standard curve prepared from the Eu standard solution provided from the vendor, Wallac. The Eu concentration was 12 µM.

The calculated stoichiometry was 1.4 Eu chelate per MCP-1 molecule.

C. Preparation of Compounds for Dispensation onto Cells

The compounds were provided as a dried film on a polystyrene "Master Plate" containing 1 µg of compound per well.

The compounds were dispensed into cell plates by preparing a 3X stock solution in binding buffer from the dried films. The cell plates were prepared according to the following method.

Prior to the day of dispensation of compounds onto cells, the "Master Plates" were allowed to reach room temperature. 50 µl each of 1X Binding Buffer (0.125% BSA in deionized water) and MCP-1 stock solution (40nM and 600 nM in 1X Binding Buffer) was added to each well. The plates were subsequently stored at 4° C overnight.

D. Time-resolved fluorescence (TRF) assay

Day 1

Each 96-well tissue culture plate (poly-D-lysine coated) was seeded with 40,000 cells in complete media containing 0.4 mg/ml G418 (200 µl per well). The plates were incubated at 37° C and 6% CO_2 and left overnight.

Day 2

The cells were approximately 90% confluent in the wells by Day 2. The cells were washed twice with Binding Buffer (no BSA) using 200 µl per wash. 40 µl of Binding Buffer was left behind following the second wash. To this final 40 µl. 100 µl of Binding Buffer was then added. Excess Binding Buffer from each well was removed leaving about 15 µl in each well. 15 µl of compound solution was dispensed into each well followed by 15 µl of 30 nM Eu-MCP1 working solution. The plates were then incubated at room temperature for 3 hours.

The plates were then washed twice with 150 µl of Wash Buffer (250 mM Hepes, pH 7.4; 1mM $CaCl_2$; 1.15 M NaCl in deionized water). 15 µl remained in each well following the washes. Then, the plates were washed ten times with Wash Buffer using 200 µl per wash. After the final wash, about 40 µl of Wash Buffer was left behind. To this residual Wash Buffer, 100 µl of Wash Buffer was added. The excess Wash Buffer was then removed, leaving about 15 µl behind. 100 µl of Enhancement Solution was added to each well. Time-resolved fluorescence was read by the Wallac/Victor Plate Reader 60 minutes later. Time-resolved fluoremetry measurements were taken from 50 µsec to 400 µsec.

E. Method of Identification of GPCR Interacting Compounds

Controls were included on each 96-well assay plate. The controls were (1) wells with no compounds and no unlabeled MCP1, (2) wells with no compounds and unlabeled MCP1 (13.3 nM) corresponding to the IC_{50}, and (3) wells with no compounds and unlabeled MCP1 (200 nM) corresponding to complete inhibition. Each control was set up in duplicates.

Hits were defined by compounds that reduce binding of Eu-MCP1 below the level of the 50% inhibition control containing unlabeled MCP1 at the IC_{50} concentration.

Percent inhibition was expressed by the following formula:

% inhibition = [1 - (test well cps - background cps) ÷ (no inhibition control cps - background cps)] x 100%;

where background is defined as cps from control set #3 (wells with complete inhibition) and the no inhibition control is defined as cps from control set #1 (wells with no compounds or unlabeled MCP1).

Hits showing greater than 50% inhibition were retested using other assay methods.

The results of this assay are summarized in the Tables below.

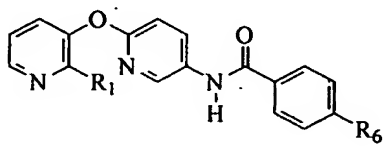
In Table 1, *** represents IC_{50}'s less than 5 μM, ** represents IC_{50}'s of between 5 and 15 μM, * represents IC_{50}'s greater than 15 μM.

In Table 2, *** represents very high binding affinity, ** represents high binding affinity, and * represents some binding affinity for the CCR10 receptor.

Table 1: Time-resolved fluorescence (TRF) assay Results for Very High Binding Affinity Compounds

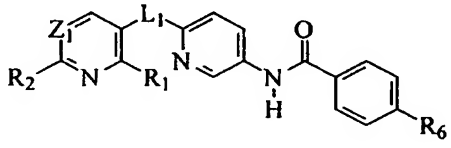
Compound	IC_{50} Rating
A	***
E	***
F	***
CU	***
CW	***
CV	***
DG	***
DO	***
EO	***
EQ	***
B	**
C	**
G	**
T	**
U	**
W	**
DR	**
DS	**
DP	**
H	*
I	*
Y	*
AB	*
DH	*

Table 2: Time Resolved Fluorescence (TRF) Assay Results



Compound #	R_1	R_6	Affinity
A	Me	CF_3	***
B	H	CF_3	***
C	H	Cl	***
E	Me	OCF_3	***
F	H	OCF_3	***
G	Me	t-Bu	***
H	H	t-Bu	***
I	Me	$CF(CF_3)_2$	***
J	H	$COH(CF_3)_2$	**
K	Me	$COH(CF_3)_2$	**
L	Me	NO_2	**
M	H	NO_2	**
N	H	$(CF_2)_2CF_3$	**
O	H	Me	*
P	H	$N(Me)_3Cl$	*
Q	Me	$N(Me)_3Cl$	*
R	H	CN	*
CV	Me	I	***
CW	Me	Br	***
CY	$CONH_2$	CF_3	*
DG	Cl	CF_3	***
DH	OMe	CF_3	***
DO	Me	Cl	***

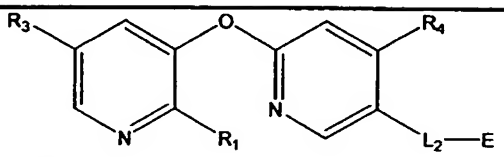
DP	Me	$CHCH_2$	***
DQ	Me	H	**
DR	Me	CH_2CH_3	***
DS	Me	$CH(CH_3)_2$	***
DT	Me	CCl_3	**
DX	Me	Me	**
EA	Br	CF_3	**
EO	Me	$S\text{-}CF_3$	***
EQ	Me	CF_2CF_3	***



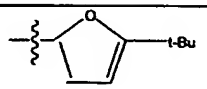
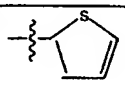
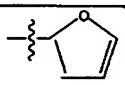
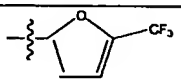
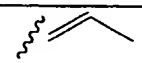
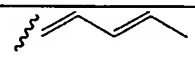
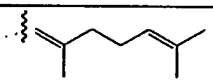
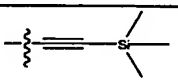
Comp.#	Z_1	R_1	R_2	L_1	R_6	Affinity
S	N	H	H	O	CF_3	**
T	CH	H	Me	O	CF_3	***
U	CH	H	Me	O	OCF_3	***
V	CH	H	Me	O	$COH(CF_3)_2$	**
W	CH	H	H	CH_2O	CF_3	***
X	CH	H	H	CH_2O	OCF_3	**
CX	N	H	H	O	OCF_3	**
CZ	N	H	H	$CH_2N(CO(p\text{-}CF_3\text{-}C_6H_4))$	CF_3	*
DA	N	H	H	CH_2NH	CF_3	*
EB	CH	I	Me	O	CF_3	*
ED	CH	H	H	NH	CF_3	**
EJ	CH	H	CH_2-Ph	O	CF_3	*

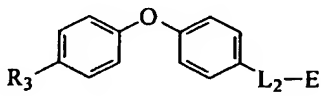
Comp.	Z_1	R_1	R_3	Z_3	L_2	R_6	R_7	R_8	Affinity
Y	CH	H	H	N	NHCO	Cl	H	H	***
Z	N	H	H	N	NHCO	Cl	H	Cl	*
CU	N	Me	H	N	NHCO	CF_3	F	H	***
AA	N	Me	H	N	NHCO	Cl	H	Cl	*
AB	N	H	H	N	NHCO	Cl	Cl	H	***
AC	N	H	H	N	$NHCH_2$	CF_3	H	H	*
AD	N	H	H	N	$NHCH_2$	Cl	H	H	*
AE	CH	H	H	N	NHCO	CF_3	H	H	**
AF	CH	H	H	CH	NHCO	Cl	H	H	*
AG	CH	H	H	CH	NHCO	CF_3	H	H	*
AH	N	H	H	N	CONH	CF_3	H	H	*
AI	N	H	H	N	$NHSO_2$	Me	H	H	*
AJ	N	H	Cl	N	NHCH (COOMe) NHCO	H	H	H	*
AK	N	H	H	N	NHCONH	H	H	H	*
AL	N	H	H	N	NHCONH	Cl	H	H	*
AM	N	H	H	N	$NHSO_2$	F	Cl	H	*
AN	N	H	Cl	N	$NHSO_2$	H	Cl	H	*
AO	N	H	H	N	$(NHCO)_2$	H	H	H	*
AP	N	H	H	N	$NHSO_2$	Cl	H	H	*
AQ	N	H	H	N	$NHSO_2$	Cl	Cl	H	*
AR	N	H	H	N	NHCO	$O(CH_2)_5$ Me	H	H	*
AS	N	H	H	N	NHCO CH_2O	H	H	H	*
AT	N	H	H	N	NHCO	H	CN	H	*

AU	CH	H	H	CH	NHCO	CF_3	H	H	*
AV	CH	H	H	CH	NHCO	Ph	H	H	*
AW	CH	H	H	CH	NHCO	$O(CH_2)_5Me$	H	H	*
AX	CH	H	H	CH	NHCO CH_2O	H	H	H	*
AY	CH	H	H	CH	NHCO	H	CN	H	*
DC	CH	H	H	CH	NHCO	t-Bu	H	H	*
DD	CH	H	H	CH	NHCO	CN	H	H	*
DE	N	H	H	N	N(Me)CO	CF_3	H	H	*
DI	N	H	COOMe	N	NHCO	CF_3	H	H	*
DJ	N	H	COOEt	N	NHCO	CF_3	H	H	*
DV	N	Me	H	N	NHCO CH_2	H	H	H	*
DY	N	H	CO-(N-piprazinyl)	N	NHCO	CF_3	H	H	*
EF	N	H	H	N	CH_2NH CO	CF_3	H	H	*

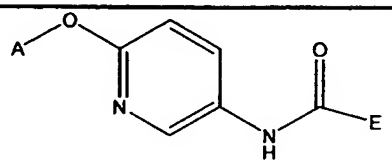


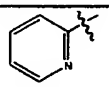
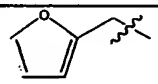
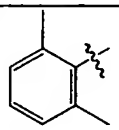
Comp	R_1	R_3	R_4	L_2	E	Affinity
BA	H	Cl	H	NHCOMe	-	*
BB	H	H	Me	NHCO	p-CF_3-C_6H_4	**
BC	H	H	Me	NHCO	p-OCF_3-C_6H_4	**
BD	H	H	H	$NHSO_2$	4-CF_3-cyclohex[1]enyl	*
BE	H	Cl	H	$NHCO(CH_2)_2COOH$	-	*
BF	H	H	H	NO_2	-	*
BG	H	H	H	$NHCH_2CH(CN)COOEt$	-	*
BH	H	Cl	H	$NCHN(Me)_2$	-	*
BI	H	Cl	H	$NHCH_2CH(CN)COOEt$	-	*
BJ	H	Cl	H	$NHSO_2Me$	-	*
BK	H	Cl	H	NHCHNOH	-	*
BL	H	Cl	H	1-pyrolyl	-	*
BM	H	Cl	H	NHCOEt	-	*
BN	H	Cl	H	NHCO-*trans*CH=CHCOOH	-	*
BO	H	Cl	H	NHCONHCO	m-Cl-C_6H_4	*
BP	H	Cl	H	NHCONHCO	2,6 dimethoxyphenyl	*
BQ	H	H	H	NHCO	3-pyridyl	*
BR	H	H	H	NHCO	3-phenyl phenyl	*
BS	H	H	H	NHCO	3-napthyl	*
BT	H	H	H	NHCO	3,5-$(CF_3)_2$-C_6H_3	*
BU	H	H	H	NHCO	cyclohexyl	*
BV	H	Cl	H	NHCO	m-Cl-C_6H_4	*

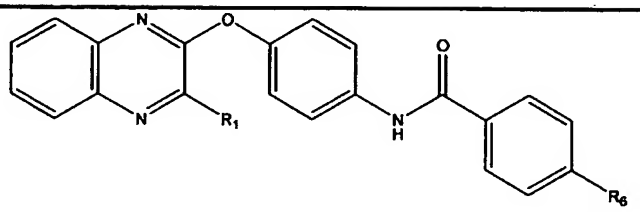
DN	H	H	H	NHCO		*
DU	Me	H	H	$NHCOCH_2$		**
DW	Me	H	H	NHCO		*
DZ	H	H	H	NHCO		*
EL	Me	H	H	NHCO		*
EM	Me	H	H	NHCO		*
EN	Me	H	H	NHCO		**
ER	Me	O	H	NHCO		*



Comp	R_3	L_2	E	Affinity
BW	H	NHCO	2-pyridyl	*
BX	H	NHCO	3-napthyl	*
BY	H	NHCO	3,5-$(CF_3)_2$-C_6H_3	*
BZ	H	NHCO	cyclohexyl	*
CA	CF_3	OCO	p-tBu-C_6H_4	*
CB	CF_3	OCO	3-pyridyl	*
CC	CF_3	OCO	(p-C_6H_5)-C_6H_4	*
CD	CF_3	OCO	3-napthyl	*
CE	CF_3	OCO	p-($O(CH_2)_5CH_3$)-C_6H_4	*
CF	CF_3	$OCOCH_2O$	phenyl	*
CG	CF_3	OCO	m-cyanophenyl	*
CH	CF_3	OCO	p-cyanophenyl	*
CI	CF_3	OCO	3,5-$(CF_3)_2$-C_6H_3	*
CJ	CF_3	OCO	cyclohexyl	*

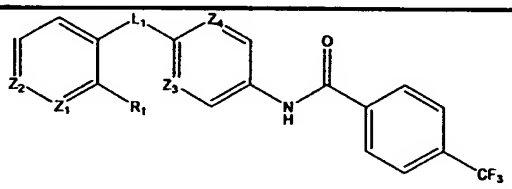


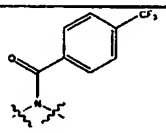
Compound	A	E	Affinity
CK	Me	p-tBu-phenyl	*
CL	Me	3-pyridyl	*
CM	Me	(p-C_6H_5)-C_6H_4	*
CN	Me	3-napthyl	*
CO	Me	p-($O(CH_2)_5$ CH_3)-C_6H_4	*
CP	Me	-CH_2-O-C_6H_5	*
CQ	Me	m-CN-C_6H_4	*
CR	Me	p-CN-C_6H_4	*
CS	Me	3,5-$(CF_3)_2$-C_6H_3	*
CT	Me	cyclohexyl	*
DF		p-CF_3-phenyl	*
DM		p-CF_3-phenyl	*
EG		p-CF_3-phenyl	**



Compound	R_1	R_6	Affinity
DB	H	CF_3	*
DL	Me	O-CF_3	*

5



Compound	Z_1	Z_2	R_1	L_1	Z_3	Z_4	Affinity
DK	CH	N	H	S	N	CH	*
EC	CH	CCl	H	CH_2	N	N	*
EH	N	CH	Me	O	N	CMe	**
EI	N	CH	H	O	N	CMe	*
EK	CH	COMe	H		N	N	*
EP	CH	COMe	H	NH	N	N	*

Example 3: Direct Binding Assay (DB Assay) for Identification of GPCR Binding Compounds

CCR10 Manual Binding Assay Method

CCR10 receptors were expressed in stably transfected K293 cells, and the cells were maintained in DMEM base medium, 10% FBS, 1X glutamine, and 0.4 mg/ml G418, and in the incubator set at 37 °C, 6.0 % CO_2 and 90% relative humidity. The day before the experiment, the cells were trypsinized and 200 µl of the cell suspension (150,000 cells/ml) was deposited into 96-well Biocoat plates (poly-D-lysine-coated). The binding assay was performed 24 hours later.

8-point dose response curves were generated as follows: compounds to be tested were dissolved in DMSO at 10 mg/ml concentration and diluted to 100 µg/ml into n-butanol. 0.75, 1.5, 3, 6, 12, 18, 24, and 30 µl of the compounds were dispensed into 8 wells of a 96-well Costar plate. In the case of more potent compounds, further dilution was applied to generate 10 µg/ml compound solutions in n-butanol, and the solutions were used to make the compound plates by dispensing 1, 2, 4, 8, 10, 20 µl of the diluted solutions, and 4 and 8 µl of the 100 µg/ml solutions, into 8 wells of the plate. The compound plates were placed in the hood overnight to evaporate the butanol, leaving dried films of the compounds. On the day of the binding assay, 50 µl of filtered binding buffer (25 mM HEPES, pH 7.4, 75 µM EDTA, 11.5 mM KCl, 115 mM NaCl, 6 mM $MgSO_4$, and 1.8 mM $CaCl_2$) was added into each well, and the plates were stored at 4 °C for about two hours. Before performing the binding assay, the compounds were thoroughly resuspended in the binding buffer.

The cells were washed three times with the binding buffer by adding and then decanting the medium or binding buffer and drying the plates on paper towels. Then the buffer was added slowly to the side wall of the wells to avoid disturbing the cells. After the third wash, the plates were dried over paper towels to leave about 5 µl of the binding buffer in each well. An additional 10 µl of binding buffer was added to each well. 15 µl of the compound solutions, and then 15 µl of the Eu^{3+}-labeled MCP-1 ligand solution

(15 nM) in binding buffer with 0.1% BSA was added. The binding reaction mixtures were maintained at RT for three hours.

After three-hour of incubation, 150 µl of the wash buffer (25 mM HEPES, pH 7.4, 0.1 mM $CaCl_2$ and 115 mM NaCl) was added into each well and the solution was decanted and the plates were dried over paper towels. Subsequently, three sets of three washes were performed. Fresh wash buffer was used at each step, and pipette tips were also changed to avoid cross-contamination of the Eu^{3+}-labeled ligand. The buffer solution of the final wash was decanted and the plate thoroughly dried on a paper towel. For each set, 100 µl of the enhancement buffer (Wallac) was added, and incubated with the cells at RT for one hour. Time-resolved fluorescence (excitation wavelength 320 nM, emission 615 nM) was measured on the Wallac Victor fluorescence reader. Inhibition of MCP-1 binding was determined according to the following formula: % inhibition = [1 - (test well cps - background cps) ((no inhibition control cps - background cps)] (100%)

Figures 1, 2, and 3 depict the binding curves for Compounds A, CU and CV, respectively.

Example 4: Cell Based Inflammatory Recruitment Assay (CBIR Assay)

THP-1 Cell Motility Assays

A THP-1 cell line was used that expresses both CCR-10 and CCR-2. The cells were placed in the top half of a chamber (Transwell plate, 24-well, 5 µM pore size, purchased from Costar) separated in the middle by a membrane. A gradient of chemokine (MCP-1 or MCP-3, purchased from R&D) was established which, in the absence of inhibitors, leads to migration of cells across the membrane. Quantitation of cell migration was done using a FACScan machine. The % migration was calculated as the number of migrated cells divided by the number of input cells. As shown in Figures 4 and 5, compounds B and C blocked chemokine induced migration of cells.

Example 5: Modulation of Recruitment of Inflammatory Cell Types using GPCR Binding Compounds B and C in a Murine Inflammatory Recruitment Assay (MIR Assay)

Mouse Peritoneal Infiltration Studies

CCR-10 and its ligands MCP-1, MCP-3 (MCP-5 in mice) have been demonstrated to mediate the recruitment of eosinophils and a variety of other leukocytes to tissue where it is expressed. Antibodies against CCR-10 have been shown to block the effects of these ligands. This example demonstrates that compounds B and C are capable of blocking MCP-5 induced peritoneal eosinophil recruitment.

Mice and in vivo procedures:

8-10-wk-old C57BL/6J mice were purchased from the Jackson laboratory (Bar Harbor, ME) and kept in Millennium Pharmaceuticals Inc. Specific Pathogen Free mouse facility.

Peritoneal recruitment assays *in vivo* with MCP-5 or mMCP-1 (mJE) protein were performed after injection of 1mg/mouse i.p. of either MCP-5 or mMCP-1. Two hours after chemokine injection, peritoneal lavage was performed and leukocytes from this organ were collected and enumerated. In one series of blocking experiments, mice were injected i.v. either with 50 nmol/Kg (7 mM/mouse) or 100 nmol/Kg (15 mM/mouse) of compound B or C 30 min before MCP-5 or mMCP-1 administration.

Immunohistochemical phenotyping and quantitation of leukocytes.

Total peritoneal cell counts were performed and aliquots (5x105 cells/slide) were pelleted onto glass slides by cytocentrifugation. To determine the number of eosinophils and mononuclear cells, slides were stained with Wright-Giemsa (Fisher Diagnostics, Pittsburgh, PA). T-lymphocytes, B-lymphocytes and mononuclear phagocytes were identified by Thy 1.2 (53-2.1) (PharMingen, San Diego, CA), IgM (II/41) (PharMingen, San Diego, CA) and Moma-2 (Biosource Int. Camarillo, CA) staining. Percentage of eosinophils, lymphocytes, neutrophils and macrophages was determined by counting their number in eight high power fields (40x magnification; total area 0.5 mm2) per area

randomly selected and dividing this number by the total number of cells per high power field. To obtain the absolute number of each leukocyte subtype in the lavage, these percentages were multiplied by the total number of cells recovered from the peritoneal fluid.

As shown in Figure 6, mice pretreated with compounds B and C prior to MCP-5 challenge showed significantly reduced levels of eosinophil recruitment than untreated mice. Control experiments with eotaxin demonstrate that the compounds are acting through CCR-10 rather than by inhibition of cytoskeletal function.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

Claims

1. A G-protein coupled heptahelical receptor binding compound wherein said compound is of the formula:

J-M

wherein

J is an aromatic moiety; and

M is a G-protein coupled heptahelical receptor pocket interacting moiety.

2. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound interacts with a β-chemokine receptor.

3. The G-protein coupled heptahelical receptor binding compound of claim 2, wherein said compound interacts with a chemokine receptor selected from the group consisting of CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, and CCR10.

4. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound modulates recruitment of at least one inflammatory cell type upon administration to a subject.

5. The G-protein coupled heptahelical receptor binding compound of claim 4, wherein said compound modulates leukocyte recruitment.

6. The G-protein coupled heptahelical receptor binding compound of claim 5, wherein said compound modulates eosinophil recruitment.

7. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound binds to a G-protein coupled heptahelical receptor with an IC_{50} of about 10 μM or less.

8. The G-protein coupled heptahelical receptor binding compound of claim 7, wherein said compound binds to a G-protein coupled heptahelical receptor with an IC_{50} of about 1 μM or less.

9. The G-protein coupled heptahelical receptor binding compound of claim 8, wherein said compound binds to a G-protein coupled heptahelical receptor with an IC_{50} of about 50 nM or less.

10. The G-protein coupled heptahelical receptor binding compound of claim 7, wherein said G-protein coupled heptahelical receptor is a β-chemokine receptor.

11. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound is an antagonist of a G-protein coupled heptahelical receptor.

12. The G-protein coupled heptahelical receptor binding compound of claim 11, wherein said antagonist is an antagonist of a β-chemokine receptor.

13. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound comprises at least one pyridyl moiety.

14. The G-protein coupled heptahelical receptor binding compound of claim 13, wherein said compound comprises two pyridyl moieties.

15. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound comprises at least one pyrimidyl moiety.

16. The G-protein coupled heptahelical receptor binding compound of claim 15, wherein said compound comprises two pyrimidyl moieties.

17. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound further comprises an amide bridging moiety.

18. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said compound further comprises an ether bridging moiety.

19. The G-protein coupled heptahelical receptor binding compound of claim 1, wherein said G-protein coupled heptahelical receptor pocket interacting moiety is a para substituted aryl moiety.

20. A compound represented by the formula:

$$A\text{-}L_1\text{-}B\text{-}L_2\text{-}E$$

wherein

A is selected from the group consisting of branched and straight chain alkyl, aryl, alkenyl, alkynyl, and heteroaryl moieties optionally substituted by NR'R", CN, NO_2, F, Cl, Br, I, CF_3, CCl_3, CHF_2, $CHCl_2$, CONR'R", S(O)NR'R", CHO, OCF_3, $OCCl_3$, SCF_3, $SCCl_3$, COR', CO_2R', and OR' and wherein R' and R" are each independently hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl or optionally substituted aryl;

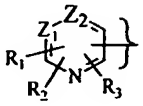
L_1 is a linker moiety selected from the group consisting of a bond, O, S, CHOH, CHSH, $CHNH_2$, CHNHR, CHNRR', NH, NR, $(CH_2)_n$, $O(CH_2)_n$, and $(CH_2)_nO(CH_2)_n$, an optionally substituted ring moiety of 4 to 7 atoms containing up to three heteroatoms, a chain of 1 to 5 atoms optionally substituted by C_1-C_6 alkyl, halogens, wherein n is either 0, 1, 2, or 3, and R and R' are each independently substituted or unsubstituted C_1-C_6 branched or straight chain alkyl, C_1-C_6 branched or straight chain alkenyl, aryl, C_4-C_7 ring, optionally substituted with up to three heteroatoms;

B is an aromatic moiety containing from 0 to 3 heteroatoms and containing 5 to 7 members optionally substituted by NR'R", cyano, nitro, halogen, CF_3, CHF_2, CONR'R", S(O)NR'R", CHO, OCF_3, SCF_3, COR', CO_2R', OR' where R' and R" are each independently hydrogen, halogen, C1-C6 alkyl, optionally substituted aryl or optionally substituted aryl;

L_2 is a second linking moiety selected from the group consisting of a bond, CH_2C=O, NHC=O, OC=O, C=O, CH_2NHC=O, CHOH, $(CH_2)_n$, O, NH, $O(CH_2)_n$, $NH(CH_2)_n$, CH_2CHOH and NRC=O; and

E is a G-protein coupled heptahelical receptor pocket interacting moiety.

21. The compound of claim 20, wherein A is represented by the following formula:



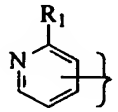
wherein

Z_1 and Z_2 each independently represent N or C;

R_1, R_2, and R_3 are independently selected from the group comprised of hydrogen, C_1-C_6 branched or straight chain alkyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl.

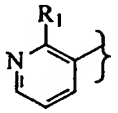
22. The compound of claim 21, wherein Z_1 and Z_2 are both carbon.

23. The compound of claim 22, wherein A is represented by the following formula:



24. The compound of claim 23, wherein R_1 is methyl.

25. The compound of claim 23, wherein A is represented by the following formula:



26. The compound of claim 25, wherein R_1 is alkyl.

27. The compound of claim 26, wherein R_1 is methyl.

28. The compound of claim 25, wherein R_1 is a halogen.

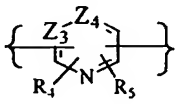
29. The compound of claim 28, wherein R_1 is chlorine.

30. The compound of claim 25, wherein R_1 is alkoxy.

31. The compound of claim 20, wherein L_1 is selected from the group consisting of S, NH, and CH_2.

32. The compound of claim 20, wherein L_1 is O.

33. The compound of claim 20, wherein B is represented by the following formula:



wherein

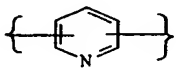
Z_3 and Z_4 each independently represent N or C;

R_4 and R_5 are independently selected from the group consisting of hydrogen, C_1-C_6 branched or straight chain alkyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl.

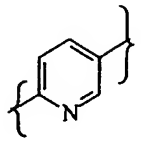
34. The compound of claim 33, wherein B is a substituted or unsubstituted pyridyl moiety.

35. The compound of claim 33, wherein B is a substituted or unsubstituted pyrimidyl moiety.

36. The compound of claim 33, wherein B is represented by the following formula:

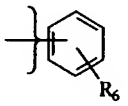


37. The compound of claim 34, wherein B is represented by the following formula:



38. The compound of claim 20, wherein L_2 is NHC=O.

39. The compound of claim 20, wherein E is represented by the formula below:



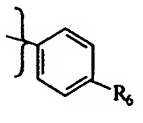
wherein R_6 is an electron withdrawing moiety and the aryl ring is additionally optionally substituted with zero to four halogen atoms.

40. The compound of claim 39, wherein E is substituted with at least one fluorine atom.

41. The compound of claim 40, wherein E is substituted with two fluorine atoms.

42. The compound of claim 39, wherein R_6 is alkyl, alkoxy, haloalkyl, nitro, halo, alkylamino, hydroxyalkyl, thioether or carboxyl.

43. The compound of claim 39, wherein E is represented by the formula below:



44. The compound of claim 43, wherein R_6 is unsubstituted alkyl.

45. The compound of claim 43, wherein R_6 is a substituted alkyl moiety.

46. The compound of claim 43, wherein R_6 is a fluorinated alkyl moiety.

47. The compound of claim 46, wherein R_6 is perfluorinated.

48. The compound of claim 47, wherein R_6 is trifluoromethyl.

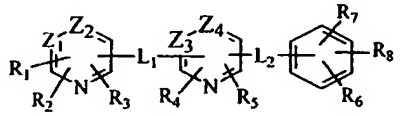
49. The compound of claim 20, wherein E is heterocyclic.

50. The compound of claim 49, wherein E is substituted or unsubstituted furanyl.

51. The compound of claim 20, wherein E is branched or straight chain, substituted or unsubstituted alkenyl.

52. The compound of claim 20, wherein E is branched or straight chain, substituted or unsubstituted alkynyl.

53. A compound represented by the formula below:



Z_1, Z_2, Z_3, and Z_4 are each independently N or C;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, C_1-C_6 branched or straight chain alkyl, alkenyl, alkynyl, alkoxy, thioalkyl, hydroxyalkyl, halo, haloalkyl, amino, alkylamino, or carboxyl;

L_1 is O, S, NH, NR_7, $(CHR_7)_n$, CO, CR_7OH, $O(CHR_7)_n$, and $(CHR_7)_nO(CHR_7)_n$ wherein n is either 1,2, or 3;

L_2 is a second linking moiety selected from the group consisting of a bond, $CH_2C=O$, NHC=O, OC=O, C=O, $CH_2NHC=O$, $NHC=OCH_2$, CHOH, $(CH_2)_n$, O, NH, $O(CH_2)_m$, $NH(CH_2)_m$, CH_2CHOH and NRC=O, wherein m is 0, 1, 2, or 3.

54. The compound of claim 53, wherein Z_1 and Z_2 are both carbon.

55. The compound of claim 54, wherein R_1 is a halogen, alkyl or alkoxy, and R_2 and R_3 are both hydrogen.

56. The compound of claim 53, wherein L_1 is O.

57. The compound of claim 53, wherein R_4 and R_5 are both hydrogen.

58. The compound of claim 53, wherein L_2 is NHC=O.

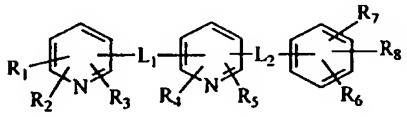
59. The compound of claim 53, wherein R_6 is a substituted or unsubstituted alkyl moiety.

60. The compound of claim 59, wherein R_6 is perhalogenated.

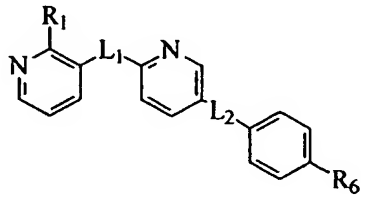
61. The compound of claim 60, wherein R_6 is trifluoromethyl or pentafluoroethyl.

62. The compound of claim 53, wherein R_7 and R_8 are each independently fluorine or hydrogen.

63. A compound of claim 53, represented by the structure below:



64. A compound of claim 63, represented by the structure below:



65. A compound of claim 64, wherein L_1 is O.

66. A compound of claim 64, wherein L_2 is NHC=O.

67. A compound of claim 64, wherein R_6 is a halogenated alkyl, alkoxy, thioether, or a halogen.

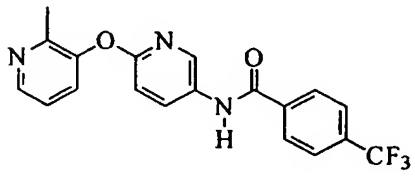
68. A compound of claim 67, wherein R_6 is trifluoromethyl or pentafluoroethyl.

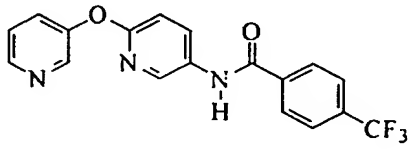
69. A compound of claim 64, wherein R_6 is a substituted alkoxy group.

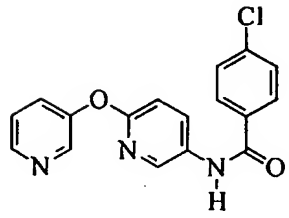
70. A compound of claim 69, wherein R_6 is a trifluoromethoxy group.

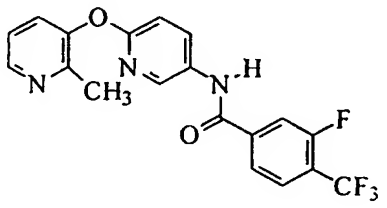
71. A compound of claim 69, wherein R_6 is a trifluoromethyl thioether group.

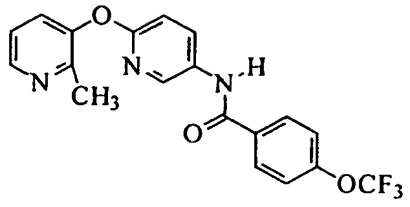
72. A compound of claim 53, selected from the group consisting of:

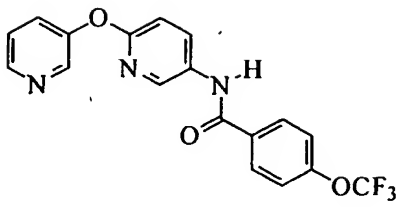


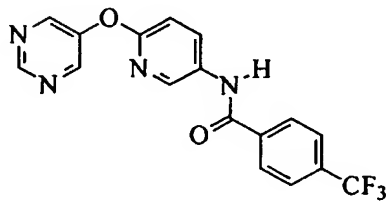


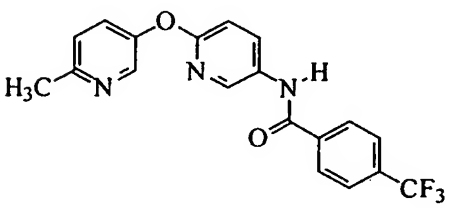


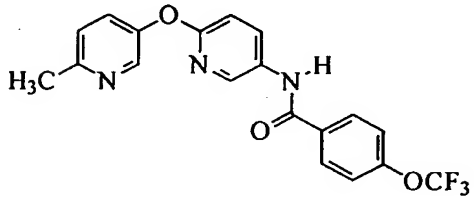


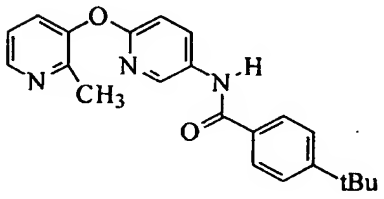


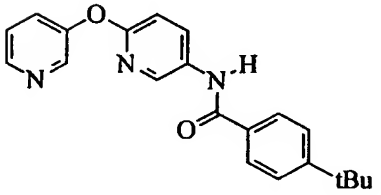


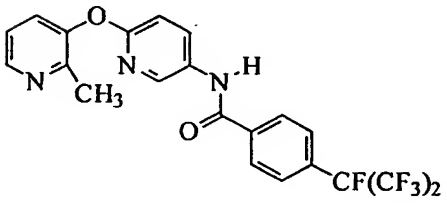


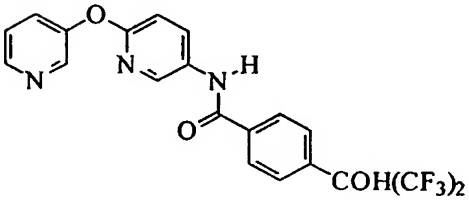


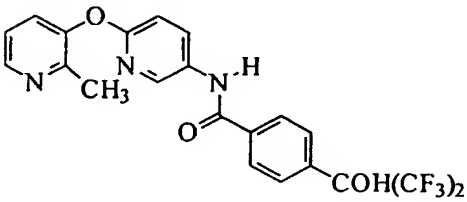


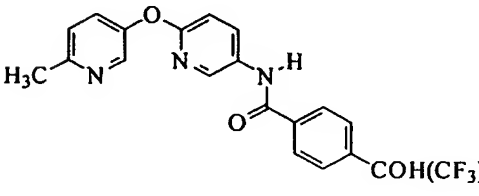


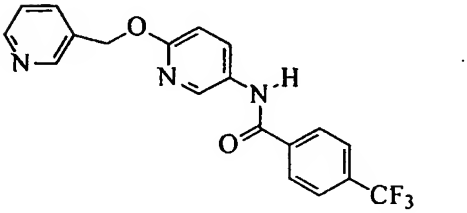


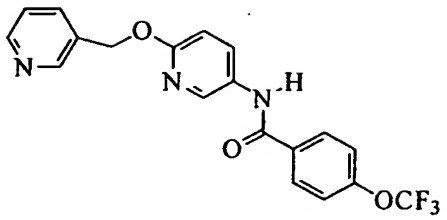


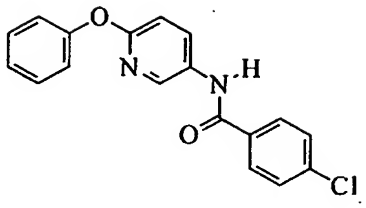


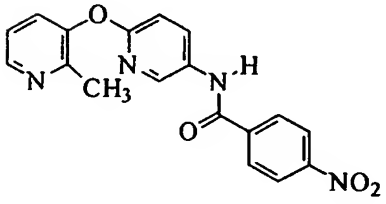


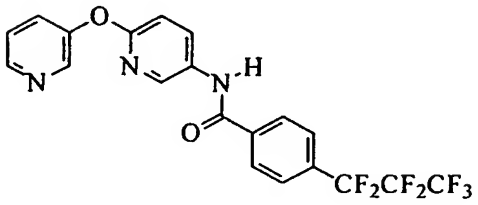


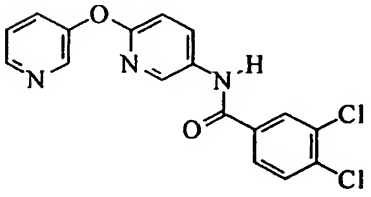


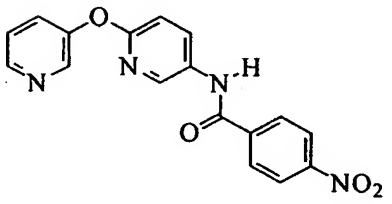


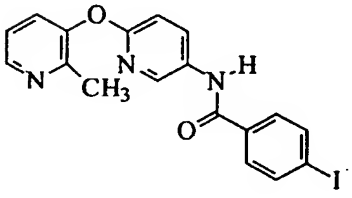


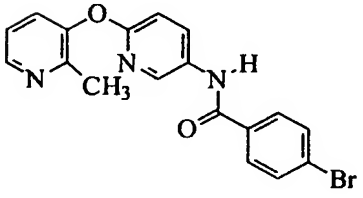


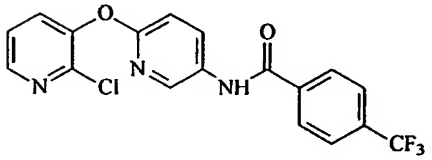


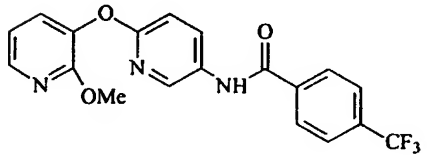


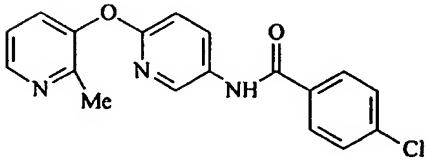


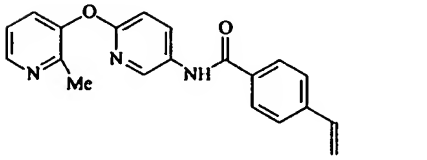


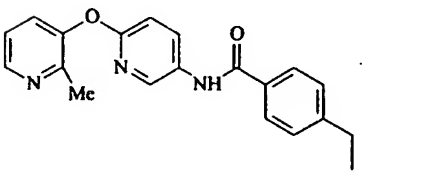


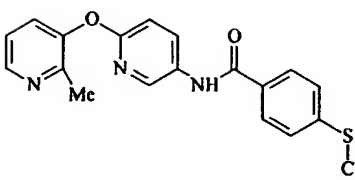


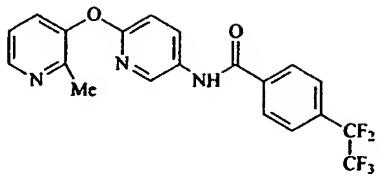


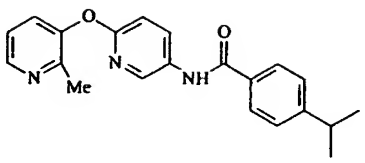












73. A method for treating a chemokine mediated disorder in a subject afflicted with said disorder, comprising

administering an effective amount of a G-protein coupled heptahelical receptor binding compound such that said disorder is treated, in that at least one symptom of the disorder is diminished or alleviated, wherein said compound is of the formula:

J-M

wherein

J is an aromatic moiety; and

M is a G-protein coupled heptahelical receptor pocket interacting moiety.

74. The method of claim 73, wherein said disorder is treated through modulation of a β-chemokine receptor.

75. The method of claim 74, wherein said β-chemokine receptor is selected from the group consisting of CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, and CCR10.

76. The method of claim 73, wherein said disorder is a neurological disorder.

77. The method of claim 76, wherein said neurological disorder is selected from the group consisting of Alzheimer's disease, dementias related to Alzheimer's disease, Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, and Jakob-Creutzfieldt disease.

78. The method of claim 76, wherein said neurological disorder is associated with inflammation.

79. The method of claim 76, wherein said neurological disorder is selected from the group consisting of stroke, traumatic injury to the brain, traumatic injury to the spinal cord, spinal crush, and central and peripheral nervous system trauma.

80. The method of claim 73, wherein said disorder is a immunological disorder.

81. The method of claim 80, wherein said immunological disorder is selected from the group consisting of immune thyroiditis, hyperthyroidism, type I diabetes mellitus, insulin related diabetes, Addison's disease, autoimmune oophoritis, autoimmune orchiitis, autoimmune hemolytic anemia, paroxysmal cold hemoglobinuria, autoimmune thrombocytopenia, autoimmune neutropenia, pernicious anemia, autoimmune coagulopathies, myasthenia gravis, allergic encephalomyelitis, pemphigus and other bullous diseases, rheumatic carditis, Goodpasture's syndrome, postcardiotomy syndrome, rheumatoid arthritis, keratitis, parotitis, polymositis, dermatomyositis, and scleroderma.

82. The method of claim 80, wherein said immunological disorder is AIDS.

83. The method of claim 80, wherein said immunological disorder is lupus.

84. The method of claim 80, wherein said immunological disorder is multiple sclerosis.

85. The method of claim 73, wherein said disorder is characterized by inflammation.

86. The method of claim 85, wherein said disorder is asthma.

87. The method of claim 73, wherein said disorder is characterized by unwanted cellular proliferation.

88. The method of claim 87, wherein said disorder is cancer.

89. The method of claim 73, wherein said disorder is characterized by unwanted cellular migration.

90. The method of claim 73, wherein said disorder is characterized by abnormal cellular signal transduction.

91. The method of claim 73, wherein said disorder is characterized by abnormal amounts of chemokine stimulated chemotaxis.

92. A pharmaceutical preparation comprised of an effective amount of a G-protein coupled heptahelical receptor binding compound and a pharmaceutically acceptable carrier, wherein said compound is of the formula:

J-M

wherein

J is an aromatic moiety; and

M is a G-protein coupled heptahelical receptor pocket interacting moiety.

93. The pharmaceutical preparation of claim 92, wherein said effective amount is effective to treat a β-chemokine mediated disorder.

94. The pharmaceutical preparation of claim 93, wherein said effective amount is an effective amount to treat asthma.

95. A packaged G-protein coupled heptahelical receptor binding compound comprising a G-protein coupled heptahelical receptor binding compound packaged with instructions for using said compound for treating a β-chemokine mediated disorder, wherein said compound is of the formula:

J-M

wherein

J is an aromatic moiety; and

M is a G-protein coupled heptahelical receptor pocket interacting moiety.

96. The packaged G-protein coupled heptahelical receptor binding compound of claim 95, wherein said β-chemokine mediated disorder is asthma.

97. A method of using a G-protein coupled heptahelical receptor binding compound, comprising using said G-protein coupled heptahelical receptor binding compound to modulate the binding of a second compound to a G-protein coupled heptahelical receptor, wherein said G-protein coupled heptahelical receptor binding compound is of the formula:

J-M

wherein

J is an aromatic moiety; and

M is a G-protein coupled heptahelical receptor pocket interacting moiety.

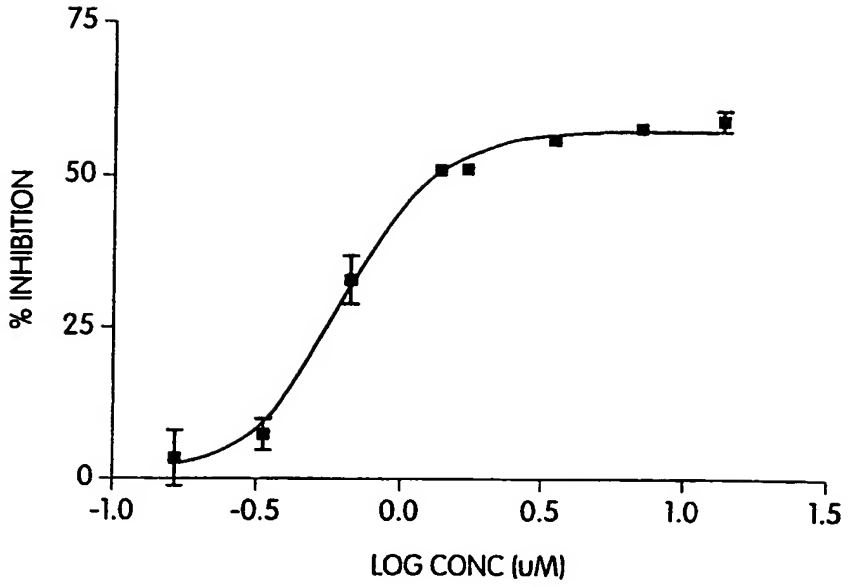

75
50
25
0
% INHIBITION
-1.0
-0.5
0.0
0.5
1.0
1.5
LOG CONC (uM)

Fig. 1

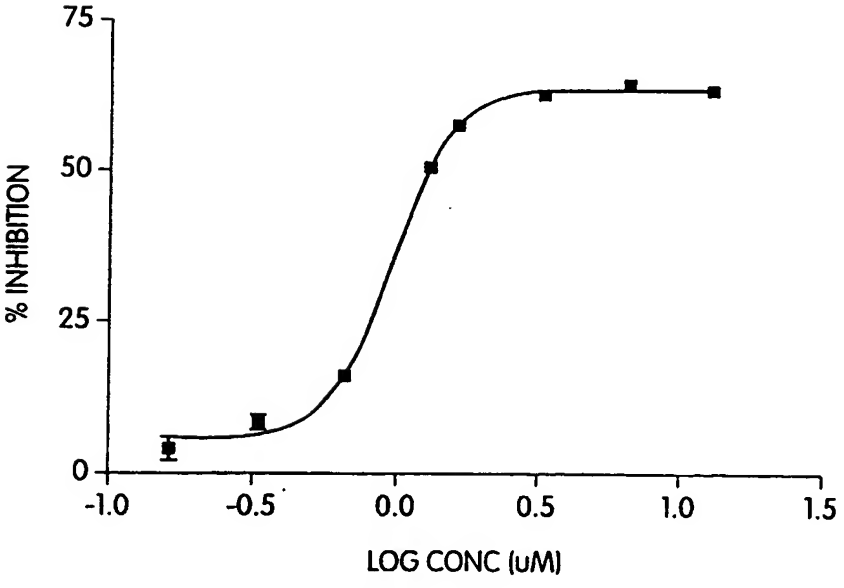

75
50
25
0
% INHIBITION
-1.0
-0.5
0.0
0.5
1.0
1.5
LOG CONC (uM)

Fig. 2

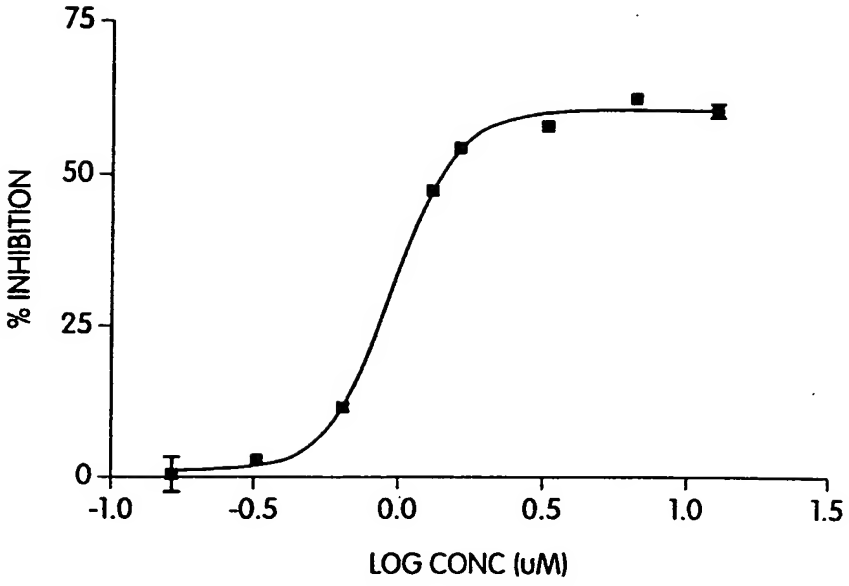

75
50
25
0
% INHIBITION
-1.0
-0.5
0.0
0.5
1.0
1.5
LOG CONC (uM)

Fig. 3

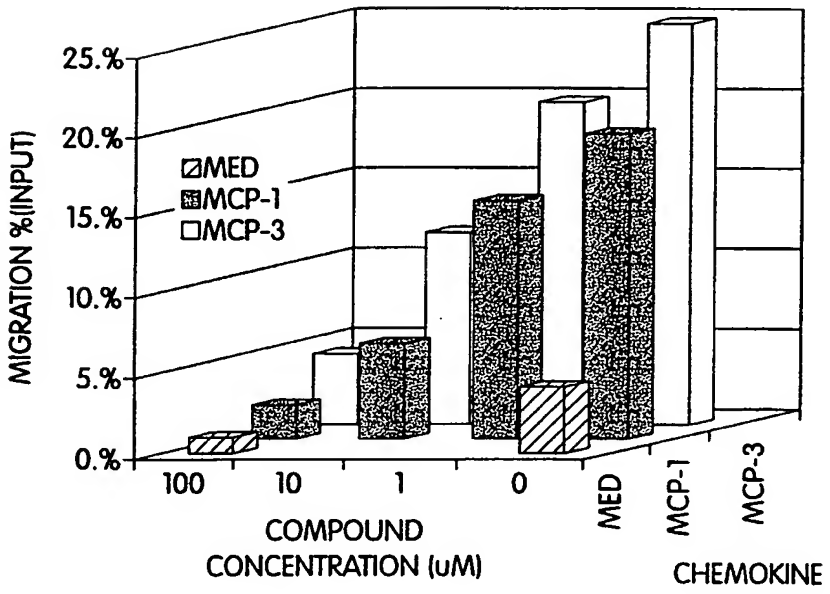

25.%
20.%
15.%
10.%
5.%
0.%
MIGRATION %(INPUT)
MED
MCP-1
MCP-3
100
10
1
0
COMPOUND
CONCENTRATION (uM)
MED
MCP-1
MCP-3
CHEMOKINE

Fig. 4

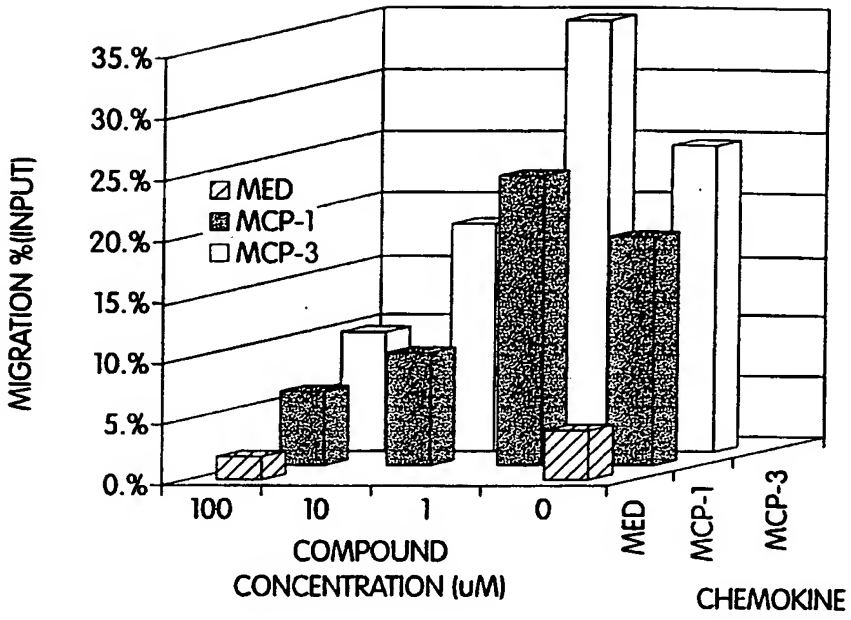

MIGRATION %(INPUT)
35.%
30.%
25.%
20.%
15.%
10.%
5.%
0.%
MED
MCP-1
MCP-3
100
10
1
0
COMPOUND CONCENTRATION (uM)
MED
MCP-1
MCP-3
CHEMOKINE

Fig. 5

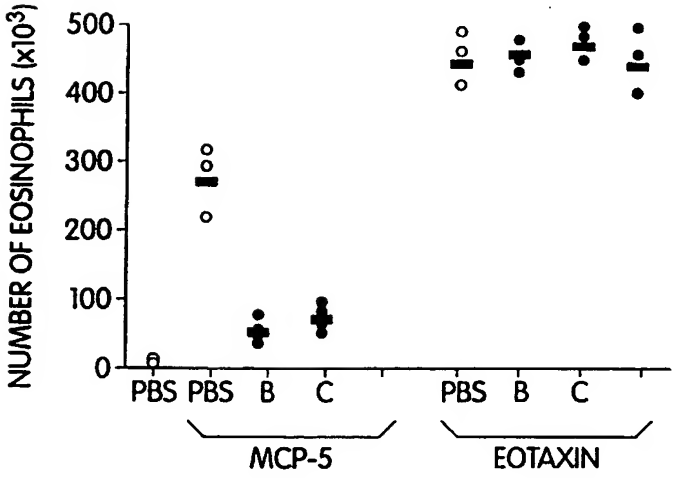

NUMBER OF EOSINOPHILS (x10³)
500
400
300
200
100
0
PBS
PBS
B
C
PBS
B
C
MCP-5
EOTAXIN

Fig. 6